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SYNDROME CHARACTERIZED BY GALACTORRHEA, AMENORRHEA AND LOW URINARY FSH: COMPARISON WITH ACROMEGALY AND NORMAL LACTATION*†

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THE combination of amenorrhea and persistent lactation, not associated either with a recent pregnancy or with acromegaly, has been sporadically noted in the past (1–5).[1] Nevertheless, it was brought to our attention afresh by a patient who came to the Ovarian Dysfunction Clinic of the Massachusetts General Hospital nine years ago.

Miss I.R., M.G.H. #374091, was an unmarried factory worker aged 31. She stated that she had been well, except for a congenitally dislocated hip, until the age of 25.

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[1] Since publication of the abstract of this article (6) additional cases have been called to our attention by Dr. K. R. Crispell (7) and Dr. Robert H. Barter (8); and the syndrome has been reported by Argonz and del Castillo (9).

Her periods started at 12 and occurred every thirty to forty days, with cramps. After the age of 25, she began to skip periods. This had happened more and more frequently until menstruation had stopped, a few months previously. When she was 27 years old she noted slight soreness in the right breast and a milky discharge from the nipple. The discharge seemed to be more marked just before a menstrual period, but was always present in some degree. Two other complaints dated from about the time her periods became irregular, namely, 1) an excellent appetite, with a tendency to gain weight, and 2) a growth of hair on her face and chest, sufficient to require the use of a depilatory once a week.

Physical examination showed a healthy appearing, rather obese young woman with a ruddy, slightly oily complexion and seborrheic dermatitis of the scalp. There was a slight growth of blond hair on the upper lip and chin. There was an easily expressible milky discharge from both breasts, more marked on the right. There was nothing about the features or the hands and feet to suggest acromegaly. On pelvic examination the uterus was thought to be small and the ovaries were not felt.

The laboratory data are shown in Table 1. Note that: 1. The serum phosphorus level was definitely lower than that in cases of acromegaly with amenorrhea. 2. The urinary FSH (follicle-stimulating hormone) assay was negative for 6.5 mouse units (m.u.) per twenty-four hours, at which level normal subjects invariably show a positive reaction. 3. The urinary 17-ketosteroids were higher than average for the patient's age, but were not outside the normal range. 4. The rate of growth of axillary hair was 12 mg. per axilla per week, whereas the normal rate is about 8 mg. per week. 5. The results of the glucose tolerance test and the insulin tolerance test, although not quite normal, did not show the insulin resistance of acromegaly or the hypoglycemia unresponsiveness of pituitary insufficiency. X-ray examination of the skull showed a normal pituitary fossa.

This patient's decreased estrin production was demonstrated by a very scant menstrual flow following one course of progesterone injections, and failure to flow following

TABLE 1. PATIENT I.R. (AGE 31)

Urinary FSH		Negative for 6.5 m.u./24 hrs.
Urinary 17-ketosteroids		10.6 mg./24 hrs.; 11.1 mg./24 hrs.
Axillary hair		12 mg./axilla/week
Serum phosphorus		3.8 mg./100 ml.; 3.3 mg./100 ml.
Blood sugar (mg./100 ml.)		
Glucose tolerance test:	Fasting	111
	30 minutes	174
	60 minutes	174
	120 minutes	141
	180 minutes	123
Insulin tolerance test:	Fasting	92
	20 minutes	55
	30 minutes	57
	45 minutes	62
	60 minutes	66
	90 minutes	85
	120 minutes	96

another course. The ovaries were visualized by culdoscopy; they were normal in size, smooth, and appeared sclerotic.

For most of the past eight years the patient has received estrin in four-week or five-week courses followed by short rest periods, during which she menstruates. She invariably reports that the discharge from her breasts diminishes while she receives estrin, and increases when estrin is stopped.

Since studying the first patient with this syndrome we have encountered about 20 more in this clinic.[2] The clinical and laboratory findings in 14 of these, as well as in our first patient, are summarized in Table 2. Note that: 1. Nine of the 15 patients had never been pregnant. 2. Two of these 9 had pseudocyesis at the onset, according to the history. 3. Some had irregular periods, but most had amenorrhea. 4. The onset in almost all patients was in their early twenties. 5. Almost all had some degree of obesity, hirsutism and seborrhea. 6. The urinary excretion of FSH was less than normal in all but 1 patient. 7. Lack of endogenous estrin was proved in the majority of instances by failure to menstruate following administration and withdrawal of progesterone. 8. The 17-ketosteroid excretion was a little above average for the patient's age in all but 2 instances. One of these 2 patients had a complicating thyrotoxicosis and the other had ceased to lactate before the assay was performed. 9. The serum phosphorus level was within normal limits in all patients, with the exception of the one who had ceased to lactate before the study. 10. In 8 of the 15 patients there was evidence of a pituitary tumor, and in 3 the tumor was proved by biopsy to be a chromophobe adenoma.

DISCUSSION

The incidence of pituitary tumor in these cases makes it very probable that the syndrome is due to a pituitary dysfunction, even where no tumor is demonstrable. It seems to us that there are at least two points of view to be considered about the cause of the lactation. A chromophobe adenoma may produce a hormone which causes lactation (presumably prolactin), or else it may interfere with the production of one of the normal pituitary hormones, the presence of which inhibits lactation. That normal pituitary function is interfered with is shown by the lack of urinary FSH and the resulting lack of estrin production by these patients. We immediately think of the amenorrhea of normally lactating recent mothers. In a few of our patients the syndrome appeared to date from a normal lactation which had failed to stop.

Normal lactation. We have studied 9 normal nursing mothers who had amenorrhea. In 1 of these, failure to menstruate following administration

[2] Ovarian Dysfunction Clinic, Massachusetts General Hospital.

TABLE 2. CLINICAL AND LABORATORY FINDINGS IN 15 CASES OF PERSISTENT LACTATION

Patient & M.G.H. No.	Age (yrs.)	Age at onset (yrs.)	Obstet. parity	Duration of lactation	Menses	Pounds over-weight	Hirsutism	Skin	FSH m.u./24 hrs.	Progesterone withdrawal bleeding	Urinary 17-KS (mg./24 hrs.)		Serum Phos-phorus (mg./100 ml.)	Pituitary status
											Observed	Normal for Age		
* I.R. 374091	39	25	0	14 yrs.	Irreg. 5 yrs. Absent 9 yrs.	27	mild, blond	Oily; red cheeks	Neg., 6.5	1. + 2. 0	10.6	6.0	3.6	Sella normal. Visual fields normal
M.P. 373037	34	26	0	12 yrs.	Absent 14 yrs.	22	slight, dark	Mild seborrhea	Neg., 6.5	—	9.0	6.0	3.7	Chromophobe adenoma
M.G. 656442	37	21	0	16 yrs.	Absent 16 yrs.	35	mild, blond	Oily; red cheeks	Neg., 6.5	0	10.7	6.0	3.3	?Enlarged sella
L.H. 288468	37	16	0	21 yrs.	Absent 21 yrs.	8.5	slight	—	Neg., 10 per 100 cc. (not elevated)	0	11.3	6.0	3.3	Chromophobe adenoma
S.M. Pvt. Pt.	39	24	0	15+ yrs.	Irreg. 1 yr. Absent 18 yrs.	6	0	Normal	Neg. 6.5	0	13.4	5.9	3.2	?Enlarged sella
S.A. 488498	57		3	20 yrs. (after tubal pregnancy)	Absent 14 yrs.	139	moderate	Seborrhea	Neg., 3.0	—	5.0	5.0	3.6	Sella normal
V.B. 507067	31	24	0	7 yrs.	Absent 7 yrs.	60+	0	Seborrhea	Pos., 6.5 Neg., 13.5	—	—	—	—	—
A.F. 729511	41		4	6 yrs. (postpartum)	Absent 6 yrs.	23	slight	Seborrhea	Neg., 6.5	0	7.2	5.9	3.4	Enlarged sella; bitemporal hemianopsia
L.M. 666443	30		0	"recent' pseudocyesis	Absent 8 yrs.	32+	slight	—	Weakly pos., 13.0	—	—	—	—	Sella normal
Z.J.* 725822	29		2	4 yrs. (postpartum)	Absent 4 yrs.	31	0	Normal	Neg., 6.5	—	—	—	3.5	Enlarged sella
S.D. 606457	26		1	1 yr. (postpartum)	Absent 1 yr.	0	mild	Normal	Neg., 3.0	+	16.4	10.0	4.3	Sella normal
J.R. 725055	26		3	2 yrs. (postpartum)	Irreg. 8 mos.	2	0	Normal	Pos., 104	Biopsy showed secretory endometrium	5.6	10.0	—	Sella normal; thyrotoxicosis
E.C.	40		2	3 yrs. (postpartum)	Absent 3 yrs.	2	0	Seborrhea	?Pos., 6.5 Neg., 13.0	0	10.3	6.0	—	Sella normal; thyrotoxicosis
E.H. 732047	47	26	0	21 yrs. (ceased 5 yrs. previously)	Absent 26 yrs.	18+	slight	Mild seborrhea	Neg., 6.5	—	4.4	5.9	4.2	Chromophobe adenoma
B.G. 755746	34	30	0	4 yrs.	Absent 4 yrs.	0 (normal)	0	Slight acne	Pos., 6.5 Neg., 13	0	—	—	3.7	Enlarged sella. Loss of vision, rt. eye

* Permission to include this case was given by Drs. Janet MacArthur and Howard Uhlfelder, who first studied the patient.

of progesterone indicated lack of estrin. Assays for FSH were performed in these mothers every two weeks, beginning two weeks after delivery. The results are shown in Table 3. Note that none of the results fell below the normal range and a few assays gave values which were above it. In fact, the FSH excretion averaged somewhat higher than in a series of non-lactating mothers similarly studied. Their data are also shown in Table 3.

Clearly, normal lactation is not due to absence of pituitary FSH. Can it be due to absence of estrin? It is not due to that alone, since neither

TABLE 3. RESULTS OF URINARY FOLLICLE-STIMULATING HORMONE (FSH) ASSAY IN NORMAL NONLACTATING AND LACTATING MOTHERS

Week		2		4		8		12		16		20	
FSH (m.u./24 hrs.)		Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
	Li.			6.5	52	6.5		6.5		26	52		
	Sh		6.5		6.5				6.5				
Non-lactating	Mi.	6.5	52		6.5								
	B	6.5	52			52	104	6.5	52				
	K	6.5	52			26	52			26	52		
	P	52	104	6.5	26	26	52						
	Gi.	6.5	52	26	52	26	52	6.5	26	26	52	13	26
	Go.	52	104			26	52	6.5	26			13	26
	C	6.5	52	6.5	52	104		13	52				
Lactating	D*	52	104		26	6.5		26	52				
	Si.	52	104	26	52	26	52	6.5	26	6.5	26		
	Mc			6.5	52	6.5	52	26	52	26		26	
	E	104		6.5	52	26	52	6.5	52	26	52		
	La	104	208	52	104			6.5	52	104		52	104
	F	104	208	52	104	52	104	26	52			26	52

* Menses recurred five weeks post partum.

the menopause nor castration cause lactation. In fact lack of estrin cannot even be a condition for lactation, since some nursing mothers have regular periods and even become pregnant. In our first patient lactation preceded amenorrhea. Estrin treatment decreased, but never entirely stopped the secretion from the breasts.

We return to the theory that the pituitary in these patients is making a specific hormone in excess. This theory can be supported as follows:

1. Lactation occurs in acromegaly, in which condition there is known to be an overproduction of hormone by the eosinophilic cells. It occurs even in cases in which the production of FSH by the basophilic cells has not been interfered with.

2. Cowdry (10) separated cells by their morphology into those which are going to be eosinophilic and those which are going to be basophilic. According to Harvey Cushing (11) the cells in chromophobe adenomata are always pre-eosinophilic in type.

3. Apart from the low urinary FSH, our patients showed no signs of pituitary failure; in fact, some of the findings such as hirsutism and elevated 17-ketosteroid excretion would be consistent with hyperpituitarism.

SUMMARY

We have presented the findings in 15 non-acromegalic women who had persistent lactation and amenorrhea associated with low urinary FSH excretion. Seven of them had a pituitary tumor. We tentatively propose that all of them had overproduction of prolactin by the pituitary.

A control study was made of FSH excretion following childbirth in 15 women, 9 of whom were nursing their babies and 6 of whom were not. All but 1 of the nursing mothers had amenorrhea. In neither group was FSH excretion decreased.

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PRELIMINARY EXPERIENCE IN TREATMENT OF THE MENOPAUSE WITH TACE, A NEW TYPE OF ESTROGEN

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PHARMACOLOGIC studies in animals (1, 2, 3) indicate that TACE[1] behaves in some respects like other estrogens but also possesses a number of unique properties. Specifically, it is absorbed following oral administration and then accumulates in the body fat, from which it is liberated gradually in ever decreasing quantities for a substantial period of time (3). In animals, the pituitary enlargement, which is a characteristic result of the repeated administration of other estrogens, does not occur (3). Similarly, effects upon the adrenal and ovary mediated through the pituitary are much less pronounced than with other estrogens (3). Clinical use of this latter phenomenon has been reported (4) in the palliative treatment of prostatic carcinoma. The administration of TACE does not appear to be complicated by the formation of adrenal androgens, as may happen during the usual hormone therapy of this condition.

Because of the prolonged and gradually decreasing estrogenic stimulation following a single course of TACE therapy, it seemed possible that advantages might be found in the treatment of the menopause. By producing a very gradual decrease in the estrogenic stimulation over a period of a month or two, women might be able to make a physiologic adaptation to their ultimate postmenopausal state so that only minimal hormone therapy might be needed. This is in contradistinction to a simple replacement therapy which may, in many instances, simply postpone the ultimate withdrawal symptoms associated with the menopause. Moreover, in cancer studies in which large doses were given to male patients (4) and to female patients (5), TACE was very well tolerated; and it was reasonable to assume that even if many of the patients would ultimately require a second or third course of therapy, it would still be preferable to attempt to produce this physiologic adaptation in as many patients as possible.

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[1] TACE® is the trade name of the Wm. S. Merrell Company, Cincinnati 15, Ohio, for its brand of chlorotrianisene. The name TACE is derived from the initials in the chemical name, Tri-para-anisylchloroethylene.

MATERIAL

The 53 patients in this study were white female private patients between the ages of 27 and 54. Their complaints were such that they each volunteered a request for therapy of menopausal symptoms. Twenty-seven of these patients were undergoing a naturally occurring menopause; 10 of the 27 had received some other form of estrogenic hormone therapy. Twenty-one of the patients had undergone a surgical menopause; of these, 6 had previously received other estrogen therapy. All of the patients requested therapy on their return postoperative visits. Five patients, 2 of whom had previously received estrogenic hormone substitution therapy, requested treatment following x-ray irradiation and radium irradiation for squamous-cell carcinoma of the cervix. None of this last group showed any evidence of recurrence of the carcinoma and none was treated with TACE until at least a period of one year had transpired since her irradiation therapy. Of the 53 patients, 51 complained of hot flushes. The 2 patients who did not have hot flushes responded poorly to treatment, which was not surprising, since neither of them presented the usual menopausal syndrome. In fact, in 1 of these instances the subsequent diagnosis was involutional melancholia and the other patient was a very unstable person with a history of previous hospitalization for schizophrenia. Nevertheless, they have been included in all analyses.

METHOD OF STUDY

The 53 patients were started on a course of TACE therapy consisting of a 12-mg. capsule of TACE in oil administered twice daily for the first two weeks, and once daily for the last two weeks, making a total of forty-two capsules (504 mg.) of the drug. Following this, therapy was discontinued until such time as the patient was re-evaluated either on a return visit or by questionnaire contact. Fourteen additional courses of TACE therapy were administered, but the data could be used in only the 11 instances in which sufficient time had elapsed since omission of the drug for symptoms to recur. This report, then, concerns 64 courses of TACE therapy in 53 patients. All patients were followed, either by questionnaire or by personal visits. The maximum duration of observation was twenty months and the minimum, three months. No additional psychotherapy, sedation, or other methods of treatment for the menopausal syndrome were used.

Of the 64 courses of therapy, 52 were carried out with TACE capsules. A short trial of TACE tablets seemed to be less satisfactory and further work with this dosage form was abandoned.

The amenorrheal index, the technique of which has been described by Kupperman *et al.* (6), was used recently in 2 patients. In both, uterine

bleeding began normally at ninety-six and one hundred and two hours following the administration of 100 mg. of progesterone.

RESULTS

Figure 1 shows the results of TACE therapy, in terms of the relief of symptoms. Twenty patients had only one symptom, hot flushes. One patient had nine symptoms. The variety of symptoms makes accurate analysis of the results more difficult, but it is apparent that the hot flushes

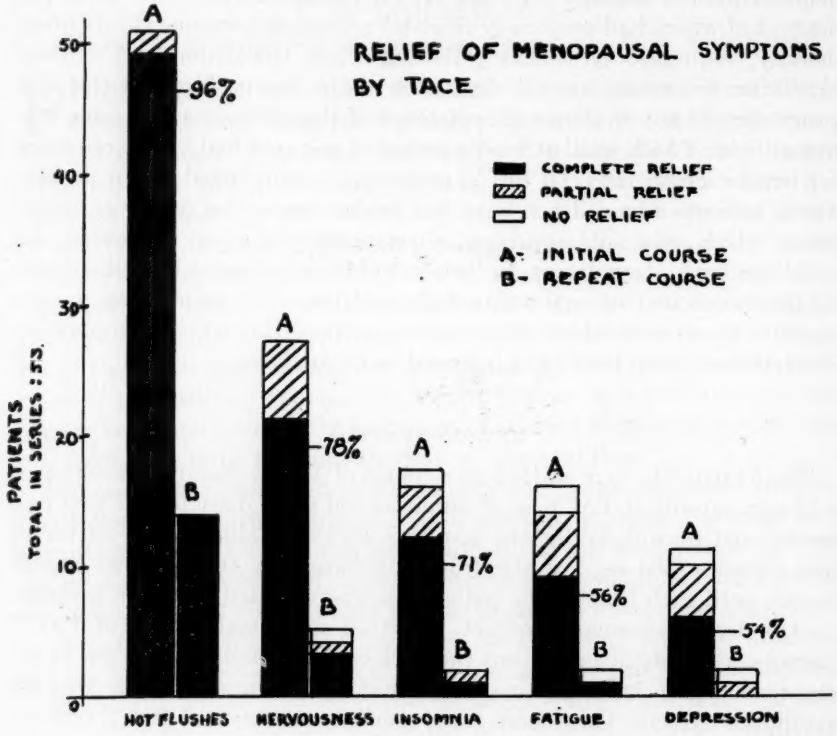

FIGURE 1

were effectively relieved by TACE; other symptoms were less effectively relieved.

Hot flushes were relieved in 49 of 51 patients who had this symptom. Of these, 37 either did not require or did not seek further treatment during the period of observation, which varied from three to twenty months.

Fourteen patients had a return of hot flushes which required a second course of treatment; the flushes were all relieved by a second course. The

average duration of relief in the group was originally tabulated as 3.8 months. This figure was arrived at by determining the time at which the patients returned for treatment or wrote requesting it. It is apparent that this is a much longer period of time than is usually noted between courses of estrogens, and does not necessarily reflect the actual duration of effect of the drug. We later had the patients note the first date of a recurrent hot flush, however mild, and in 10 of 16 patients the duration of the symptom-free period was twenty-three days, with a range of seven to forty-three days.

Eighteen patients had received other forms of estrogen treatment for the menopausal symptoms (diethylstilbestrol in 8, mixed estrogens in 6, ethinyl estradiol in 2, and an unstated type in 2). In Figure 2 the results are compared with those following the administration of TACE. Using the

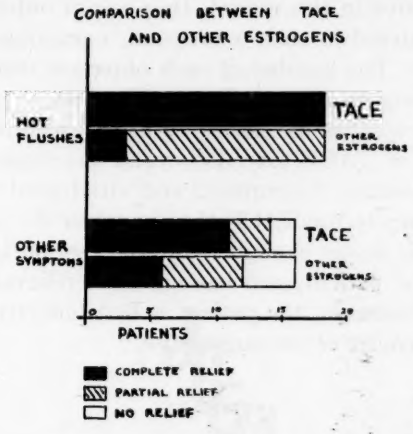

FIGURE 2

patients' statements regarding the effectiveness of the therapy in relieving symptoms, TACE was superior in effect in 15 of the 18 patients, equally effective in 3 and inferior to other estrogens in none.

Four patients were given placebos. One reported no effect; in another there was lessening but persistence of symptoms. A gradual diminution of symptoms to complete disappearance was reported by a third patient, and the fourth stated that all symptoms except hot flushes were completely relieved.

Four patients had vaginal smears taken before and after treatment. These smears all showed a change from an atrophic state before therapy to a full-blown estrogen effect after TACE administration.

Three endometrial biopsies prior to treatment showed a resting and early

proliferative phase of the endometrium. At the conclusion of therapy 2 patients showed what would be estimated to be a normal tenth-day proliferative endometrium; the third showed no demonstrable difference between initial and post-therapy biopsies.

Side effects were uncommon. Only 2 patients experienced withdrawal bleeding. This was of a heavy nature due to endometrial hyperplasia, and curettage was necessary. Bleeding occurred at seventeen days and thirty-three days following completion of therapy. Edema of the legs occurred in 1 patient in whom it had also occurred with two other estrogen preparations.

DISCUSSION

This study was established primarily to determine the effect of TACE on the subjective symptoms of the menopause. The results appear to show that TACE is effective in this regard. However in order to eliminate the errors often encountered in such a program, more objective criteria are obviously necessary. The number of such objective studies has been too few to permit drawing definite conclusions. To date, they simply bear out the estrogen-like properties of TACE, with the exceptions as noted. Apparently the effect of TACE extends beyond the conclusion of therapy, judging by delayed return of symptoms and withdrawal bleeding.

It is now our policy to use TACE therapy when the menopausal symptom of hot flushes is severe enough to interfere seriously with the rest or daily routines of the patient, and has not been relieved by mild phenobarbital sedation. However, the patient is first indoctrinated and understands the natural course of the menopause.

SUMMARY

1. TACE, a new synthetic estrogen, has been employed in the treatment of severe menopausal symptoms in 53 private patients.

2. Forty-nine of the 51 patients complaining of hot flushes were completely relieved of this symptom.

3. The side effects were few.

4. Eighteen patients had previously received other hormone therapy. In 3 of these, the effectiveness of TACE was equal to that of the other hormones, and in 15 it was superior. In no instance was TACE less effective than other estrogens.

5. We recommend a further trial and more thorough evaluation of TACE, on the basis of the effectiveness of its control of menopausal symptoms in this preliminary study on 53 patients.

6. No contraindications have been noted to its judicious use in appropriate patients with severe menopausal symptoms.

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EFFECT OF INTRAVENOUS CALCIUM GLUCONATE UPON THE EXCRETION OF CALCIUM AND PHOSPHORUS IN PATIENTS WITH IDIOPATHIC HYPOPARATHYROIDISM*

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PATIENTS who have a serum calcium level below 7 milligrams per 100 milliliters are known to have a greatly reduced urinary calcium excretion. The availability of 3 patients with low serum calcium values due to idiopathic hypoparathyroidism offered an opportunity to observe the effect of raising the serum level on the rate of urinary excretion of calcium. For this purpose, calcium gluconate was administered intravenously. We wish to present our observations on the changes in calcium excretion under these circumstances.

MATERIAL AND METHODS

A 3 per cent solution of calcium gluconate was prepared in the laboratory and 500 milliliters, containing approximately 1,350 milligrams of calcium, were injected intravenously at the rate of approximately 4 ml./min. Each study was performed in the morning, with the patients in the postabsorptive state. During the administration of the calcium, venous blood samples were drawn from the opposite arm at intervals of approximately one-half hour. Urine samples were obtained through an indwelling catheter at the intervals indicated in Tables 1–3. The bladder was washed out with 20 ml. of physiologic salt solution after each period, and complete emptying was assured by the injection of air into the bladder.

Creatinine was determined by the method of Bonsnes and Taussky (1), and phosphorus by a modification of the method of Fiske and SubbaRow (2). Serum calcium was determined by the method of Van Slyke and Sendroy (3). Urinary calcium was assayed by permanganate titration of the oxalate (4), on the urines of Patients KMT and GY, and by the method of Kochakian and Fox (5) on the urine of Patient EAG. The urinary calcium was measured for Patient EAG, only in specimens which demonstrated a positive Sulkowitch test. Serum protein was determined by the method of Kingsley (6), and estimates of the ionized calcium were based on the tables of McLean and Hastings (7). In order to correlate serum levels with the urinary levels during specific collection peri-

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ods, it was assumed that there was a three-minute delay from the time of filtration until the appearance of the urine in the bladder.

Patient KMT was a 28-year-old white male school-teacher, whose case history has been more completely described in a previous publication (8). He first became aware of hypoparathyroid tetany at the age of 10 years, but had failed to follow a consistent therapeutic regimen. Physical examination showed him to be normal, except for the surgical absence of teeth because of defective development, and a persistently positive Chvostek reaction. **Patient GY** was a 54-year-old white male, in whom the diagnosis had been made only a short while before, although he had had evidence of tetany for twenty years and had undergone bilateral removal of cataracts at the age of 41. He had received one course of therapy with AT-10, but the drug was withdrawn prior to study. **Patient EAG** was a 56-year-old white salesman, who had also had symptoms for over twenty years, and in whom cataracts had been removed at the age of 35. His diagnosis had been established about two years previously, and his symptoms had subsequently been concontrolled with ergosterol. His therapy was also withdrawn prior to study. All 3 patients had characteristic blood changes (Tables 1–3), normal or low-normal renal function, increases in bone density and intracranial calcifications by roentgen examination, and prolonged P-R intervals on the electrocardiogram. In other respects they were essentially normal for their respective ages.

RESULTS

Intravenous administration of calcium gluconate resulted in elevation of the serum calcium level in all cases. Increases ranged from 4.3 milligrams per 100 milliliters to 7.8 milligrams, averaging 5.5 milligrams. The highest final value was 13.0 milligrams per 100 milliliters, obtained during treatment of KMT (Fig. 1, Table 1). Despite the rate of administration, there were no untoward effects except for a chill during the second study of Patient EAG, which was followed by a slight fever. It was not determined whether this was due to the preparation of the solution, or to the rapid rate of administration, which in this patient was 6 milliliters per minute. No subsequent ill effects were noted.

Only trace amounts of calcium were excreted when the serum calcium concentration was below a critical level, which varied somewhat from patient to patient. Patient KMT (Fig. 1, Table 1) demonstrated a tenfold increase in the rate of calcium excretion when the serum calcium concentration exceeded 7.8 milligrams per 100 milliliters, a value equivalent, at a level of 7.25 grams of protein per 100 milliliters, to 3.2 milligrams of ionized calcium. For Patient GY (Fig. 2, Table 2) the critical values were 6.7 and 2.7 milligrams per hundred milliliters, and for EAG (Table 3) they appeared to be about 8.5 and 3.7. Since these values are the result of extrapolation, first from a changing plasma level determined over a period of time defined by the appropriate urine collection period, and then from the McLean-Hastings nomogram, they must be considered to be approximations only. Once calcium appeared in the urine, further increases were fairly directly related to increases in the plasma level of ionized calcium.

TABLE 1. PATIENT KMT

(Serum protein 7.25 Gm./100 ml.; creatinine 1.00 mg./100 ml.)

Time	No. of mins.	Urine vol. (ml.)	Glom. filt. rate (ml./min.)	Serum Ca (mg. /100 ml.)	Serum ionized Ca (mg. /100 ml.)	Urine Ca (mg. /min.)	Serum P (mg. /100 ml.)	Urine P (mg. /min.)
March 7, 1951—Ca gluconate (500 ml. of 3% solution), 10:27 a.m. to 12:55 p.m.								
a.m.								
9:40								
10:00	20	86	127	5.0	2.1	.05	5.8	.98
10:20	20	116	114	4.7	1.9	.07	5.7	.68
10:36	16	141	113	5.2	2.2	.02	4.5	.78
10:53	17	184	109	5.5	2.3	.03	4.2	.71
11:12	19	246	115	5.7	2.4	.02	4.4	.63
11:32	20	154	127	6.2	2.6	.04	4.4	.56
11:47	15	141	104	7.9	3.3	.44	4.6	.87
p.m.								
12:09	22	229	119	9.2	3.9	.68	5.7	1.09
12:29	20	292	111	10.0	4.3	.94	6.6	1.44
12:58	29	266	108	10.7	4.7	1.19	6.6	1.57
March 21, 1951—Ca gluconate (500 ml. of 3% solution), 10:08 a.m. to 12:15 p.m.								
a.m.								
9:03								
9:25	22	30	114	5.5	2.2	.01	6.7	.66
9:45	20	45	150	5.9	2.4	.01	6.7	.96
10:08	23	160	134	6.3	2.6	.01	6.7	.74
10:29	21	375	113	6.8	2.8	.03	6.7	.79
10:52	23	385	102	7.8	3.2	.02	6.6	.78
11:12	20	124	100	8.7	3.6	.25	7.0	.77
11:35	23	295	103	9.9	4.2	.48	7.0	1.11
11:55	20	438	108	11.5	4.9	.51	6.9	1.10
p.m.								
12:15	20	300	94	13.0	5.6	1.13	7.4	1.21
12:41	26	138	105	12.1	5.2	.89	7.7	1.12
12:58	17	69	109	11.2	4.7	.66	8.0	.85
1:32	34	52	96	10.5	4.4	.37	7.6	.74
2:25	53	40	104	9.5	4.0	.14	7.0	.58
3:26	61	42	109	9.2	3.8	.13	6.5	.90

Despite the large amounts of calcium administered, in no case was more than 10 per cent recovered in the urine during the period of observation, and the quantity of calcium in the urine started to decline immediately after the

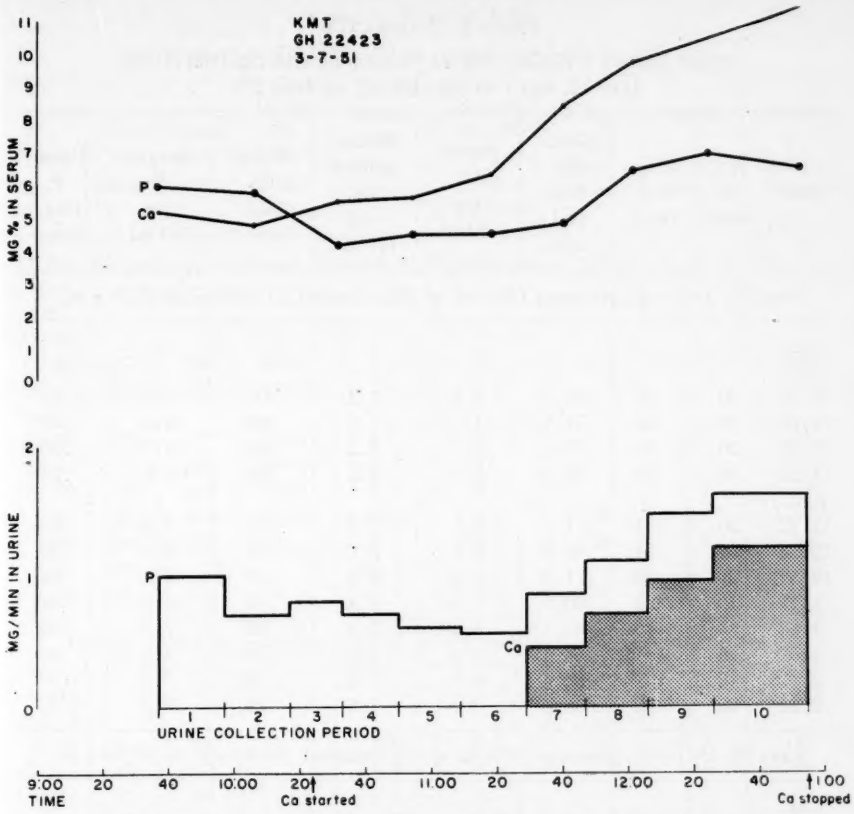

FIG. 1. Calcium and phosphorus changes in the serum and urine of Patient KMT during first infusion of calcium gluconate.

cessation of the infusion. Several observations two or more hours after this point indicated that the serum level was still significantly elevated, and calcium excretion still well above control levels. However, since the peak rate of excretion had passed, it is doubtful that the final total excretion exceeded 25 per cent at the most.

The maximum rise in serum calcium concentration was 7.8 milligrams per 100 milliliters, and was observed in the second study on Patient KMT. The minimum rise was 4.3 milligrams per 100 milliliters, observed during the first study of Patient EAG. This marked rise in the calcium level despite the large volume of distribution throughout the extracellular compartment and the relatively slow rate of injection, suggested that the removal of the excess calcium was relatively slow.

An unexpected observation was the increase in the serum phosphorus

TABLE 2. PATIENT GY

(Serum protein 7.25 Gm./100 ml.; creatinine 1.46 mg./100 ml. on June 12, and 1.80 mg./100 ml. on June 22)

Time	No. of mins.	Urine vol. (ml.)	Glom. filt. rate (ml./min.)	Serum Ca (mg./100 ml.)	Serum ionized Ca (mg./100 ml.)	Urine Ca (mg./min.)	Serum P (mg./100 ml.)	Urine P (mg./min.)
June 12, 1951—Ca gluconate (500 ml. of 3% solution), 11:38 a.m. to 2:18 p.m.								
a.m.								
10:33								
10:53	20	40	65.5	4.2	1.2	.05	6.9	.47
11:13	20	38	54.8	4.1	1.2	.05	6.9	.34
11:33	20	31	57.0	4.2	1.2	.06	6.9	.36
11:52	19	30	60.2	5.0	2.0	.05	6.9	.34
p.m.								
12:12	20	34	64.7	6.8	2.8	.10	6.9	.33
12:32	20	34	65.0	6.8	2.8	.26	6.9	.29
12:52	20	35	71.3	7.2	3.0	.47	6.9	.32
1:11	19	53	60.0	7.9	3.3	.43	6.9	.41
1:32	21	85	61.1	8.6	3.6	.32	7.3	.45
1:52	20	146	56.4	8.9	3.8	.73	7.8	.65
2:12	20	152	62.4	8.8	3.8	.73	8.3	1.92
2:32	20	62	58.0	8.8	3.8	.70	8.8	0.32
June 22, 1951—Ca gluconate (500 ml. of 3% solution), 10:32 a.m. to 12:20 p.m.								
a.m.								
10:37								
10:58	21	50	76.2	5.6	2.2	.06	6.2	.34
11:23	25	172	71.3	7.5	3.2	.18	6.2	.36
11:39	16	192	64.0	9.0	3.8	.36	6.2	.45
11:54	15	186	64.7	9.2	3.9	.52	6.1	.63
p.m.								
12:08	14	182	69.3	9.4	4.1	.70	6.6	.79
12:26	18	128	66.7	9.6	4.2	.78	7.4	.82
12:44	18	76	62.6	9.4	4.1	.53	7.3	.87
1:30	46	162	71.7	9.1	3.9	.47	7.4	.94
1:45	15	60	74.9	8.9	3.7	.30	7.4	.50
2:04	19	55	55.0	8.8	3.7	.31	7.2	.78

concentration which followed the rise in the serum calcium. This rise averaged 1.9 milligrams per 100 milliliters of serum, and had a range of 1.4 to 2.7 milligrams. The highest terminal level was 9.0 milligrams per 100 milliliters, seen during the first study on Patient GY. Control studies on Patients GY and EAG during the same time of day, but without cal-

TABLE 3. PATIENT EAG

(Serum protein 7.0 Gm./100 ml.; creatinine 1.35 mg./100 ml.)

Time	No. of mins.	Urine vol. (ml.)	Glom. filt. rate (ml./ min.)	Serum Ca (mg. /100 ml.)	Serum ionized Ca (mg. /100 ml.)	Urine Ca (mg. /min.)	Serum P (mg. /100 ml.)	Urine P (mg. /min.)
Feb. 12, 1952—Ca gluconate (500 ml. of 3% solution), 11:10 a.m. to 12:58 p.m.								
a.m. 11:08								
11:35	27	210	89.5	7.0	3.0	†	4.2	.39
11:56	21	155	85.2	7.6	3.2	†	4.4	.24
p.m. 12:16	20	125	75.0	8.2	3.5	†	5.7	.36
12:39	23	290	86.9	8.4	3.6	†	5.9	.99
12:59	20	250	94.0	8.7	3.8	†	5.7	1.45
1:35	36	295	66.8	10.8	4.8	.79	6.1	1.48
2:01	26	135	71.1	10.1	4.5	.89	6.6	1.35
2:24	33	*	*	10.1	4.5	*	6.5	*
2:46	22	115	72.9	10.1	4.5	.73	6.4	1.07
Feb. 29, 1952—Ca gluconate (360 ml. of 3% solution), 9:54 a.m. to 10:51 a.m.								
a.m. 8:45								
9:06	21	55		6.6	2.8	†	4.3	.45
9:25	19	55		6.6	2.8	†	4.5	.41
9:48	23	84		6.5	2.7	†	4.7	.30
10:05	17	90		6.5	2.7	†	4.9	.74
10:17	12	115		7.3	3.1	†	5.7	.85
10:27	10	130	75.0	8.5	3.7	.08	5.5	1.29
10:36	9	97	63.5	9.7	4.3	.32	5.0	1.28
10:45	9	75	76.3	10.4	4.6	.62	5.1	.94
10:57	12	63	66.5	11.0	4.9	.40	5.3	1.08

* Spilled.

† Insufficient calcium for analysis.

cium infusion, failed to show fluctuations of greater than 0.5 milligram per 100 milliliters. Elevations in the serum phosphorus concentration were associated with parallel changes in the urinary phosphorus excretion. However, these changes were not as consistently directly related as were those of calcium.

DISCUSSION

Probably less is known regarding the renal excretion of calcium than of any other important urinary constituent. It is believed that only the ion-

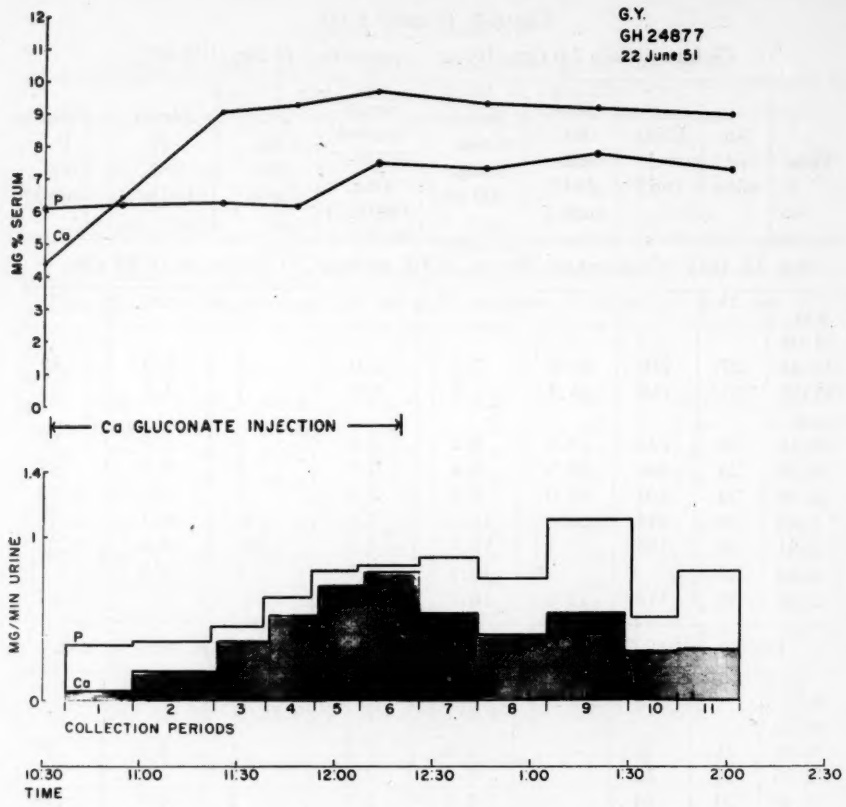

FIG. 2. Calcium and phosphorus changes in the serum and urine of Patient GY during second infusion of calcium gluconate.

ized calcium is filtered, and that the amount of calcium in the filtrate depends upon the concentration of calcium ion in serum and upon the filtration rate. Unfortunately, the ultrafiltration data of Hopkins, Howard and Eisenberg (9) show values significantly greater than the values for calcium ion concentration obtained by McLean and Hastings (7). Since there is no completely acceptable method for determining the amount of filtered calcium, the contribution of the renal tubule, either by reabsorption or by excretion, is unknown.

By using the results obtained from the creatinine clearances, and estimating the amount of ionized calcium from the McLean-Hastings tables, it is possible to obtain an approximate theoretic value for filtered calcium. By subtracting the excretion of urinary calcium per minute from the estimated filtered calcium per minute, a value for tubular reabsorption may be

proposed. Using this technic, GY was found to have a filtration rate of 65 ml./min. and an average maximum calcium reabsorption of 1.8 mg./min. Similar values for EAG were 80 ml./min. and 2.9 mg./min.; and for KMT, 110 ml./min. and 3.5 mg./min. Ratios for the values GFR/TM (Ca) were 36, 28 and 31 respectively.

An abnormal hypercalciuria exists when the calcium in the urine exceeds 300 milligrams per day. This rate would be achieved by the average excretion of 0.2 milligram per minute. Examination of the data reveals that when the serum calcium levels reached values of 10 milligrams per 100 milliliters, the rate of calcium excretion was on the order of 1.0 milligram per minute. Such an excretion rate would result in the urinary loss of 1,500 milligrams of calcium a day. It is therefore apparent that in the normal individual with a serum calcium level of 10 milligrams per 100 milliliters, the rate of calcium loss must be much less, and that the mechanism for calcium equilibrium cannot be the same as that observed during these acute studies. Either an increase in the reabsorptive Tm (Ca) or an alteration in the physical state of the calcium, which reduces its filterability, is the most probable explanation. It is possible that calcium-protein equilibration is slow, and that during the infusion the proportion of ionized calcium was excessively high. On the other hand, the serum concentration observed at the time at which urinary calcium appeared in significant quantity is in agreement with data on patients with hypocalcemia during treatment of hypoparathyroidism. This question should yield to direct examination more readily than would changes in Tm (Ca).

The rise in the concentration of serum phosphorus despite the concomitant increase in the urinary phosphorus is unexplained. Howard, Hopkins and Connor (10) have recently reported similar observations. It is interesting that in normal individuals receiving intravenous calcium gluconate, Howard and his co-workers demonstrated a fall in the phosphorus excretion which they attributed to the cut-off of parathyroid hormone by the rising level of serum calcium. However, even under these circumstances, the conservation of phosphorus by reduced renal excretion was insufficient to account for the increased serum levels. In hypoparathyroidism the serum phosphorus concentration rose after calcium infusion, despite the increased urinary loss of phosphate, and this clearly could not have been due to renal conservation.

SUMMARY AND CONCLUSIONS

1. Two courses of 500 milliliters of 3 per cent calcium gluconate solution were administered to each of 3 patients with idiopathic hypoparathyroidism.

2. This administration of calcium produced a marked and relatively

sustained elevation in the serum calcium level. Initially insignificant urinary calcium excretion suddenly increased when the serum calcium concentration rose above a value which was critical for a given patient, but which was somewhat variable from patient to patient. Above this critical level calcium excretion appeared to increase linearly, suggesting a reabsorptive Tm (Ca). The rate of calcium excretion under these circumstances was greater than that of a normal individual with the same serum calcium concentration, indicating that the mechanism in these patients was still not normal.

3. Following the rise in the serum calcium levels, there was a marked rise in the concentration of serum phosphorus. This response was associated with a comparable increase in urinary phosphorus excretion.

Acknowledgment

The authors wish to acknowledge the assistance of Dr. Earl Mason, who analyzed some of the urine specimens for calcium.

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FURTHER STUDIES ON THE TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA WITH CORTISONE

V. EFFECTS OF CORTISONE THERAPY ON TESTICULAR DEVELOPMENT*†

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THE effects of cortisone therapy on the sexual development of female pseudohermaphrodites with congenital adrenal hyperplasia have been discussed in previous papers (1, 2, 3). In most girls in whom treatment was begun after the age of 8 years there was rapid development of the breasts and other female sex organs, and ovulatory menstrual cycles were established promptly. In several girls 5 or 6 years of age there was slight budding of the breasts without further progress of adolescent development. In still younger children no sexual changes were noted. The onset of adolescent female development seemed to depend upon the level of somatic development, as determined by the bone age which the patient had attained before cortisone treatment, rather than upon the chronologic age. The girls over 8 years of age who showed rapid development all had bone ages over 14 years.

The present study deals with the effects of cortisone therapy on the testicular development of 7 boys with macrogenitosomia praecox due to adrenal hyperplasia, and with the effects of the removal of adrenal tumors on the testes of 2 boys who were precociously virilized.

Condition of testes before treatment

The cases of adrenal hyperplasia are listed in Chart 1. When treatment was begun, the patients varied in age from 3 years and 6 months to 8 years

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‡ Argentine Fellow of the W. K. Kellogg Foundation under the preceptorship of the American College of Physicians. Dr. Cara is now in Cordoba, Argentina.

and 9 months. They all showed accelerated growth in height, advanced osseous development and increased musculature. They all had pubic hair and marked enlargement of the penis. The output of urinary 17-ketosteroids ranged from 8.5 to 40 mg. per twenty-four hours. The degree of virility, manifested by the amount of sexual hair and other signs, was in general proportional to the level of 17-ketosteroids. In all cases the testes were of preadolescent size, the smallest measuring 2.2×1.0 cm. and the largest 2.8×1.7 cm. They were of normal consistency and no nodules were felt.

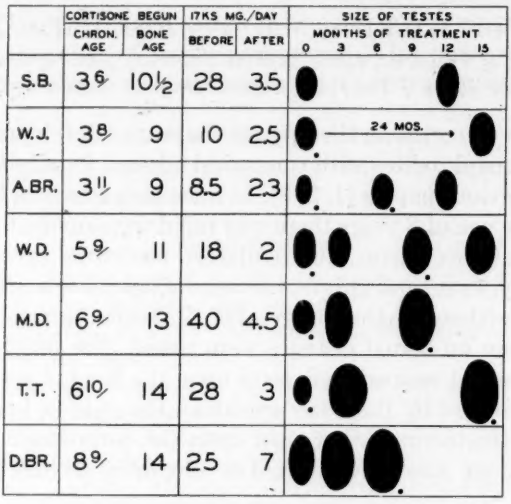
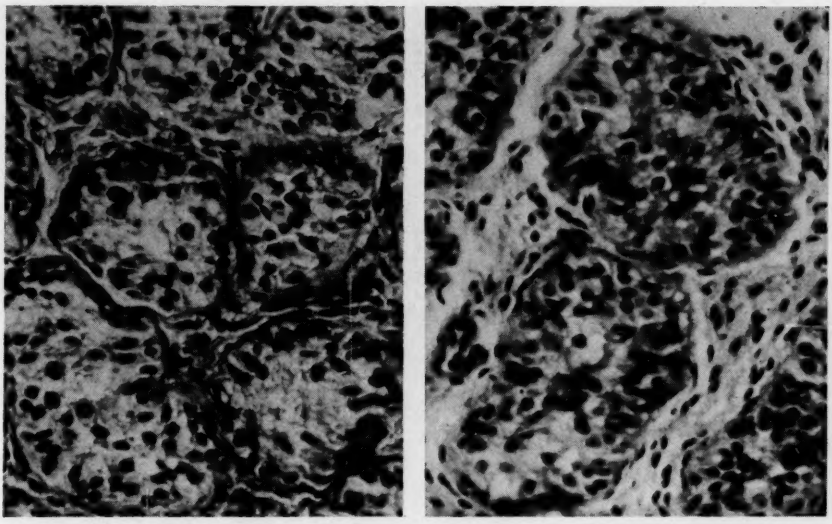


CHART 1. The effects of cortisone therapy on the 17-KS excretion and the size of the testes of 7 boys with virilism due to adrenal hyperplasia. The testes are drawn to scale. Before treatment the average measurement was 2.5×1.3 cm. Following treatment the testes of the 3 older boys increased to an average of 4.5×2.7 cm. The dots indicate testicular biopsies.

Testicular biopsies were performed on 6 of the patients (indicated by dots on Chart 1) before treatment was begun. In the younger patients (Fig. 1 A) the tubules were small and showed narrow or no lumina. They were composed of two or three layers of undifferentiated cells, but a few spermatogonia with clear protoplasm could be recognized. No mitoses were present. Sertoli cells could not be identified. There was not much intertubular tissue separating the tubules, and no Leydig cells were present. In the older patients (Figs. 2A and 3A) the tubules were larger and the lumina were more distinct and contained masses of cells with pyknotic nuclei. The walls of the tubules were thicker. More spermatogonia were present and occasional mitotic figures and spermatocytes could be recog-



A. *Untreated*
Patient A.Br.
Age, 3 $\frac{11}{12}$ yrs.
Bone age, 9 yrs.

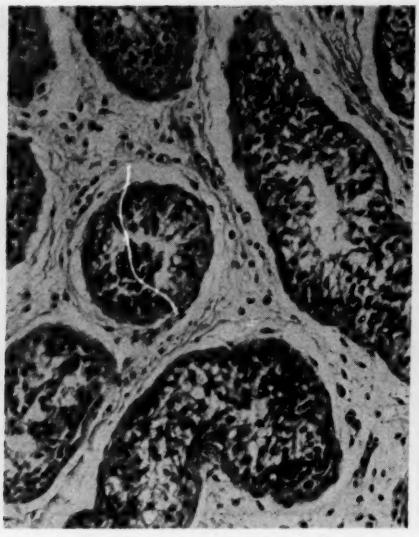
B. *Treated with cortisone for 7 mos.*
Patient A.Br.
Age 4 $\frac{6}{12}$ yrs.

FIG. 1. Testicular biopsies before and after treatment in Patient A.Br. Note the immaturity of the tubules, the presence of only occasional spermatogonia, and the absence of Sertoli cells and Leydig cells before treatment. There was no significant change following treatment except slight growth of the tubules and a little increase of undifferentiated intertubular tissue.

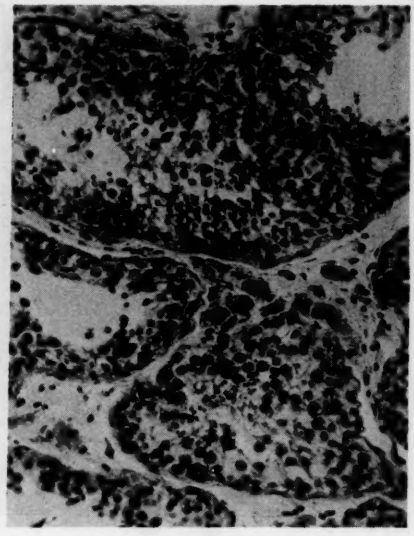
nized. In no instance were there spermatids or spermatozoa. Some Sertoli cells were differentiated. The tubules were frequently widely separated by loose undifferentiated connective tissue. No Leydig cells were present.

Effects of treatment

The effects of cortisone given in doses previously described (2, 3) are shown in Chart 1. In each case there was a marked decrease in the output of urinary 17-ketosteroids. The effects on the size of the testes varied considerably. In the first 3 patients, who were between $3\frac{1}{2}$ and 4 years of age and had bone ages between 9 years and $10\frac{1}{2}$ years, there was no significant growth of the testes during the next twelve to twenty-four months, and there was no increase in virilization during this period. A testicular biopsy performed on Patient A.Br. seven months after treatment was begun showed no significant change from the condition observed before treatment (compare Fig. 1A and 1B). The tubules showed no increase in maturation and no Leydig cells appeared.



A. *Untreated*
Patient M.D.
Age 6 $\frac{9}{12}$ yrs.
Bone age 13 yrs.

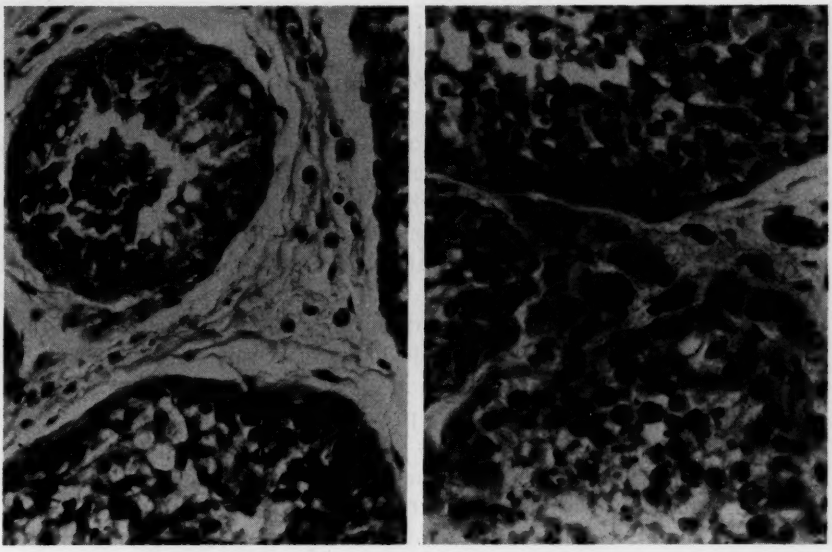


B. *Treated with cortisone for 9 mos.*
Patient M.D.
Age 7 $\frac{6}{12}$ yrs.

FIG. 2. Testicular biopsies before and after treatment in Patient M.D. Before treatment the tubules were more developed than in Patient A.Br. (Fig. 1) but there was no mitotic activity or spermatogenesis. There was more abundant intertubular connective tissue but no Leydig cells. There was marked maturation with active spermatogenesis after treatment, and Leydig cells were present.

Patient W.D. who was 5 years and 9 months old and had a bone age of 11 years when first treated, showed a slight increase in the size of his testes during the next nine months. The tubules were larger and showed numerous spermatocytes with mitoses. Some spermatids were present, but Leydig cells were not identified.

The 3 oldest patients (M.D., T.T. and D.Br.), who varied in age from 6 years and 9 months to 8 years and 9 months and had bone ages of 13 and 14 years, showed marked rapid growth of their testes within the first three to six months of treatment. The testes attained the size of approximately 4.5×2.7 cm. In all 3 cases biopsies showed maturation corresponding to that of a young adult (Figs. 2B and 3B). The tubules were large, with well defined lumina. The germ cells showed abundant mitoses with active spermatogenesis. In the intertubular tissue there were numerous Leydig cells. In contrast to the younger groups, treatment with cortisone did not seem to check the progress of the masculinization in these patients.



A. *Untreated*
Patient M.D.

B. *Treated with cortisone for 9 mos.*
Patient M.D.

FIG. 3. Higher magnification of areas in Figure 2, showing that after treatment there were numerous spermatozoa and Leydig cells (center).

Hirsutism, acne and muscular development continued to advance as they do in normal adolescents. In 1 of the patients, D.Br., the 17-ketosteroids could not be suppressed below an average level of 7 mg. per day, presumably due to the secretion of testicular androgen.

DISCUSSION

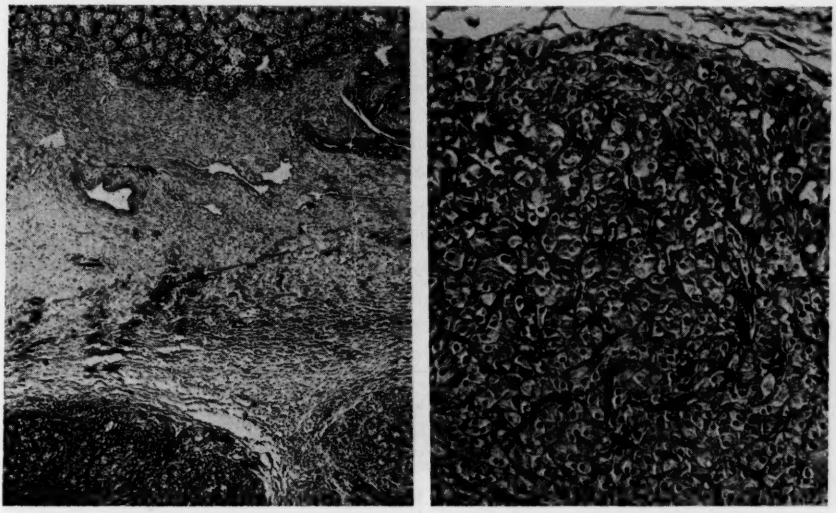
The study of the testes of untreated patients with adrenal hyperplasia confirms the findings of Sobel, Sniffen and Talbot (4). These authors examined testicular biopsy specimens in 3 patients with adrenal hyperplasia who were, respectively, 14 months, 4 years and 10 months, and 5 years and 7 months of age. The testes were of preadolescent size. Although in the older patients the tubules showed some growth with differentiation of Sertoli cells and occasionally development of germ cells to the stage of spermatocytes, there was no spermatogenesis, and Leydig cells did not appear. This was in marked contrast to the picture in cases of the constitutional type of sexual precocity in which active spermatogenesis occurred at an early age and Leydig cells were numerous.

It is probable that the large amounts of androgenic and estrogenic steroids secreted into the circulation by the hyperplastic adrenals inhibit the

secretion of pituitary FSH and LH, so that spermatogenesis and Leydig-cell development do not occur (2). On the other hand, the adrenal androgen may be responsible for the growth of the tubules, the development of the germ cells to the stage of spermatocytes and the differentiation of Sertoli cells. The inhibition of pituitary gonadotropins may persist indefinitely in an untreated case of adrenal hyperplasia. Recently, Dr. H. Nowakowski (5) of Hamburg, Germany sent us photomicrographs of a testicular biopsy specimen taken from a 25-year-old man who had untreated adrenal hyperplasia with virilization, diagnosed at the age of 5 years. The testes were small. The germinal cells showed mitoses with development to the stage of spermatocytes, but spermatids and spermatozoa were absent. No Leydig cells were seen. There was slight thickening of the basilar membrane but no other degenerative change.

Another testicular abnormality, previously reported in some cases of congenital adrenal hyperplasia, was seen in one of the 7 patients studied (W.J.). When a biopsy was performed on the left testis at the age of 22 months, a dark brown nodule about 4 mm. in diameter was noted on its surface. The entire gonad was removed and proved to be normal but immature. The nodule was composed of cells resembling those of the reticular zone of the adrenal (Fig. 4). One of the authors (6) has reported a case in which the testes were replaced almost entirely by infiltration of aberrant adrenal tissue resembling the reticular zone. The finding of adrenal tissue in the testes of patients with adrenal hyperplasia has been reported also by Skelton (7), Thelander (8), Cohen (9), Allibone *et al.* (10), and Gardner *et al.* (11). In 3 young infants with adrenal hyperplasia Landing and Gold (12) found nodules localized in the region of the testicular hilum and rete testis, which they believed to be composed of Leydig cells rather than ectopic adrenal tissue. However, in these cases no Leydig cells were found in the intertubular tissue and the testicular tubules were immature and showed no evidence of spermatogenic activity. The fact that the cells found in the testis in some cases of adrenal hyperplasia are adrenal rather than Leydig cells is borne out by a recent observation of A. Prader of Zurich (personal communication), who found that in an adult with congenital adrenal hyperplasia, whose testes were enlarged with cells of this type, cortisone therapy caused a marked decrease in size.

The growth and maturation of the testes, which occurred rapidly in the older boys after the institution of cortisone therapy, is of considerable interest. This cannot be a direct effect of cortisone on the testes, because it does not occur in patients who are treated with cortisone for other diseases. It is probable that when the excessive secretion of adrenal androgens and estrogens is suppressed by cortisone, their inhibitory effect on the pituitary is withdrawn and gonadotropins are rapidly secreted. The same



A B

FIG. 4. Testicular biopsy in Patient W.J., before cortisone treatment. A. In the upper portion there are immature seminiferous tubules and in the lower portion there is a nodule of aberrant adrenal tissue located outside of the tunica. B. High-power view of the nodule, showing resemblance to adrenal tissue.

mechanism is probably responsible for the ovarian maturation and rapid feminization which occur in older female pseudohermaphrodites treated with cortisone. In female patients, we have been able to demonstrate the absence of urinary gonadotropins before treatment and their appearance after treatment (2). Gonadotropic assays have not been made in the males.

The data presented suggest that the activation of the pituitary gonadotropic mechanism depends upon the general level of somatic maturity the patient has attained before cortisone treatment is instituted. Under the influence of excessive adrenal androgen, the height, muscular development and skeletal maturity are all far in advance of the patient's chronologic age. Probably the hypothalamic-pituitary-axis also attains precociously a level of maturity corresponding to that of puberty, the time when gonadotropic secretion normally begins. However, the actual secretion of gonadotropins is held in check by the excessive adrenal hormones until these are suppressed by cortisone. As indicated by the "bone age," the critical level of maturity which determines whether or not adolescent development begins, seems to be about 11 to 13 years. This agrees with our previous observations that in boys who are retarded in growth and epiphyseal development, sexual changes do not begin until the bone age reaches about 12 years. In contrast to our findings which suggest that in human beings a

certain level of maturity must be attained before the secretion of pituitary gonadotropin can be activated by the withdrawal of sex hormones, are the experiments of Kupperman *et al.* (13) on rats. These workers reported that when antigonadotropic serum was injected daily into rats from the tenth to the nineteenth day of life, there was suppression of the normal postnatal development of the ovary; when the antihormone was then stopped, there was a rapid and precocious development of the ovary with opening of the vagina and establishment of estrous cycles.

Observations made on 2 young boys from whom virilizing adrenocortical tumors were removed (Chart 2) help to substantiate the hypotheses previously discussed, namely: 1) that testicular maturation is not due

EFFECT OF REMOVAL OF ADRENOCORTICAL TUMOR					
	AT OPERATION		17 KS MG./DAY		SIZE OF TESTES
	CHRON. AGE	BONE AGE	BEFORE	AFTER	MONTHS 0 3 6 9 12 15
T.W.	3 10/12	5½	16	<1	3 YEARS
H.B.	4 9/12	12	360	2.5	

CHART 2. The effects of removal of virilizing adrenal tumors on the 17-KS and the size of the testes of two boys who were 3¾ years and 4¾ years old but had bone ages of 5½ years and 12 years, respectively.

directly to cortisone but depends upon suppression of the secretion of excessive adrenal androgens and estrogens, and 2) that testicular maturation occurs only when the level of maturity, as measured by the bone age, is that of about 11 to 13 years. Neither of these patients received cortisone. The younger boy was 3 years and 9 months of age and had a bone age of 5½ years when an adrenal tumor was removed from his liver (14). The urinary 17-ketosteroids fell from 16 mg. to less than 1 mg. per twenty-four hours. The testes remained small and are still immature at the age of 7 years and 6 months. All signs of virilization disappeared after operation. The other boy was 4 years and 9 months old at the time of operation. He had had a large adrenal tumor for at least two years and his bone age had reached a level of 12 years. The urinary 17-ketosteroid output, which before operation ranged from 200 to 500 mg., decreased to 2.5 mg. per twenty-four hours postoperatively. The testes, which had been small and immature, enlarged to adult size within seven months after operation. At the age of 6 years, testicular biopsy showed very active spermatogenesis and Leydig cells (Fig. 5). The boy continued to show progression of male sexual development and increasing hirsutism. There is no evidence of a

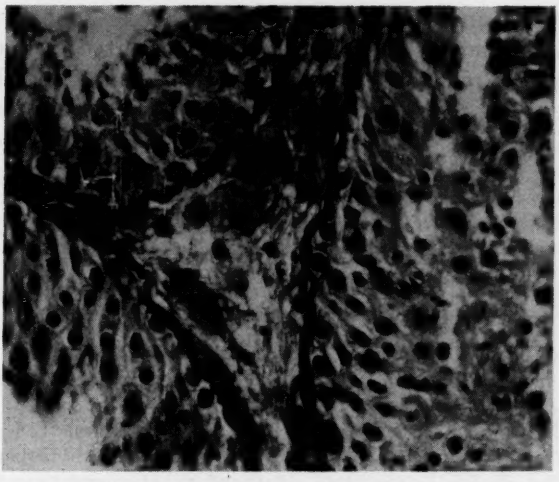


FIG. 5. Testicular biopsy in Patient H.B., fifteen months after the removal of a virilizing adrenal tumor. At the time of operation he was $4\frac{3}{4}$ years old and had a bone age of 12 years. At the time of biopsy he was 6 years old. Note the active spermatogenesis with numerous mitoses and spermatozoa and the presence of Leydig cells.

recurrence of tumor and the 17-ketosteroid output has not exceeded 2 or 3 mg. per twenty-four hours.

SUMMARY

Studies are reported on the size and histologic appearance of the testes of 7 boys with virilism due to adrenal hyperplasia, before and after treatment with cortisone. Before treatment the testes were of preadolescent size. The tubules were immature. The germ cells either had not differentiated beyond the stage of spermatogonia or showed some mitoses with development only to the stage of spermatocytes. Spermatids and spermatozoa were not found. No Leydig cells were present. With cortisone therapy, boys who were $6\frac{3}{4}$ to $8\frac{3}{4}$ years of age but had the osseous maturity of 13 to 14 years showed rapid growth of the testes with active spermatogenesis. Numerous Leydig cells appeared. In boys who had an osseous maturity of less than 11 years, no growth or maturation of the testes occurred under cortisone treatment.

It is believed that the testicular maturation is not a direct effect of cortisone but is due to the release of pituitary gonadotropins which occurs when the excessive secretion of adrenal androgens and estrogens is suppressed. The ability of the pituitary to secrete gonadotropins is attained when the general level of maturity, as measured by the bone age, is about 11 to 13 years. These hypotheses are substantiated by the fact that when a

virilizing adrenal tumor was removed from a $3\frac{3}{4}$-year-old boy with a bone age of $5\frac{1}{2}$ years, no testicular development occurred, whereas when a similar tumor was removed from a boy $4\frac{3}{4}$ years old with a bone age of 12 years, there was rapid testicular maturation with spermatogenesis.

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ADRENAL RESPONSE TO ACTH IN VARIOUS CLINICAL CONDITIONS

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IN MAN, stimulation of the adrenal cortex by ACTH results in secretion of 11-oxygenated steroids in which compound F predominates. This in turn produces an increase in the urinary excretion of metabolites of these adrenal steroids, causing an increased titer of 17-ketosteroids, formaldehydogenic corticoids and glycogenic corticoids in the urine. The urinary titer of steroids can thus be employed as a measure of the ability of the adrenal to respond to ACTH stimulation (1). It has been reported that certain adrenocortical hormones cause peripheral blood eosinopenia and an increased urinary uric acid/creatinine (U/C) ratio (1). Therefore, these changes, when measured, may be used as additional indices of adrenal function.

Various other means of determining the presence or absence of adrenal function have been suggested. These include epinephrine stimulation of the hypothalamic-anterior pituitary-adrenocortical axis, using the four-hour total blood eosinophil response as the end point (2, 3), and the more specific ACTH stimulation of the adrenal cortex with the 4-hour eosinophil and U/C changes noted (4). A 48-hour ACTH test was introduced by Thorn and Forsham (1), in which measurements of eosinophils, U/C and urinary 17-ketosteroids were used as a means of more accurately estimating adrenal response during continued ACTH stimulation. We have utilized a modification of this test in an attempt to evaluate its usefulness in determining adrenal function in a group of patients exhibiting a variety of diseases. Some of the data have already been reported (5).

METHODS

Determinations were obtained on 27 patients on the wards of Charity Hospital and Foundation Hospital in New Orleans. Patients were not specially prepared for the tests, except that all medication was discontinued prior to, and during, the period of study. No attempt was made to control diet. Uncatheterized, voided urine specimens were

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collected in clean containers. No preservative was used but the specimens were continuously refrigerated by means of bedside portable iceboxes. In each case, a twelve-hour urine specimen was collected from 8 p.m. until 8 a.m. the night before the ACTH was to be administered. Control levels of 17-ketosteroids, formaldehydogenic corticoids, and uric acid/creatinine were determined on this specimen. Following a control total blood eosinophil count at 8 a.m., 25 mg. (25 I.U.) of lyophilized ACTH was injected intramuscularly. A urine specimen was collected at 9 a.m., and discarded. A three-hour specimen was then obtained and analyzed for 17-ketosteroids and U/C. A fourth-hour total blood eosinophil count was made at noon. Beginning at 4 p.m., 10 mg. of ACTH was injected intramuscularly every six hours for seven doses. Urine was collected from the twenty-fourth to the forty-eighth hour after the initial dose of ACTH, and determinations were made of U/C, formaldehydogenic corticoids, and 17-ketosteroids. At twenty-four hours, and again at forty-eight hours, blood eosinophil levels were determined.

The total blood eosinophil counts were performed according to Henneman's modification of Randolph's technique (6). The methods employed for determinations of 17-ketosteroids, gonad-stimulating hormones, creatinine, formaldehydogenic corticoids, and uric acid, were the standard ones employed in our laboratory (7).

RESULTS

A positive adrenal response to administration of ACTH was considered to be one in which at least two of the four determinations showed significant changes. A >50 per cent increase over the control level of urinary formaldehydogenic corticoid or 17-ketosteroid excretion, a >50 per cent decrease in total blood eosinophils or a >50 per cent increase in the urinary U/C ratio are all believed to be indicative of a significant adrenal response.

Table 1 shows the analyses of 24 positive responses to the ACTH test. Although significant eosinophil, 17-ketosteroid and formaldehydogenic corticoid responses were obtained in approximately the same percentage of cases, occasionally one of these determinations was indicative of a good response when the others did not show any change. This was most often true of the eosinophils.

This "dissociated" type of response is well depicted in Figure 1. Each

TABLE 1. ANALYSIS OF 24 POSITIVE RESPONSES TO 48-HOUR ACTH TESTS*

Determination	No. of significant responses	Per cent of total
Formaldehydogenic corticoids	22	92
Eosinophils	20	83
17-Ketosteroids	19	79
Uric acid/creatinine	11	46

* Results were considered positive if two or more determinations showed significant changes.

of these 4 patients had carcinoma of the breast with far advanced metastases, although the nutritional status was rather good. In Patient A.L., the eosinophils showed a significant decrease to zero, the formaldehydogenic corticoids and U/C ratio both rose, but the 17-ketosteroids showed little change. Patient E.S. illustrated good formaldehydogenic corticoid and U/C changes, but the eosinophil and 17-ketosteroid responses were delayed, in that significant changes did not occur until twenty-four to forty-eight hours had elapsed. The third patient, J.T., showed a rather delayed

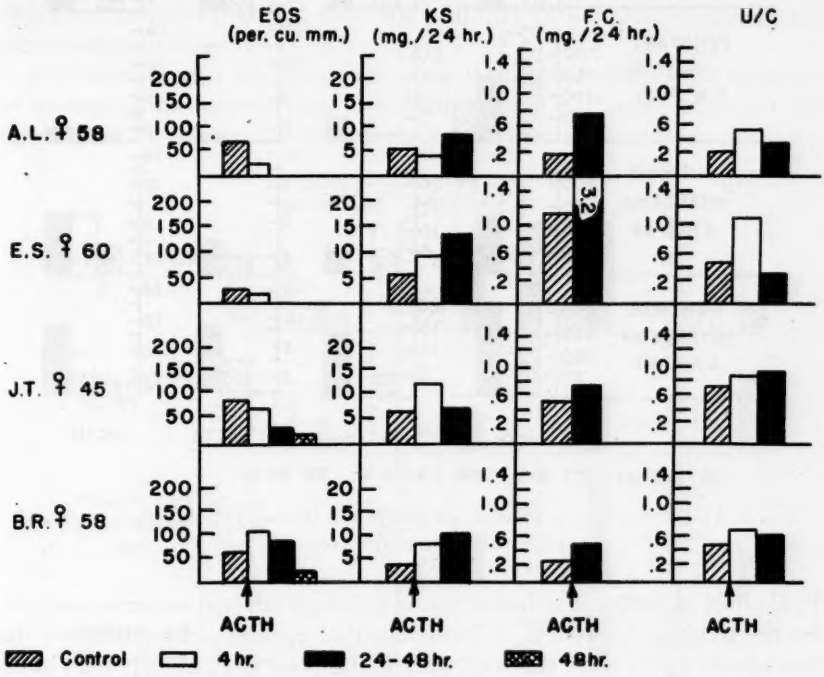

FIG. 1. "Dissociated" responses to ACTH administration in 4 patients with advanced carcinoma of breast.

eosinophil response, a good 17-ketosteroid response, but poor formaldehydogenic corticoid and U/C changes. Patient B.R. showed increases in 17-ketosteroids and formaldehydogenic corticoids, delayed eosinopenia, and little or no change in the U/C ratio.

In Figure 2 is shown a normal response (D.G.) to ACTH. There is a greater than 50 per cent decrease in the eosinophils and a marked increase in 17-ketosteroid excretion within the first four hours following administration, with a good formaldehydogenic corticoid response and a fair

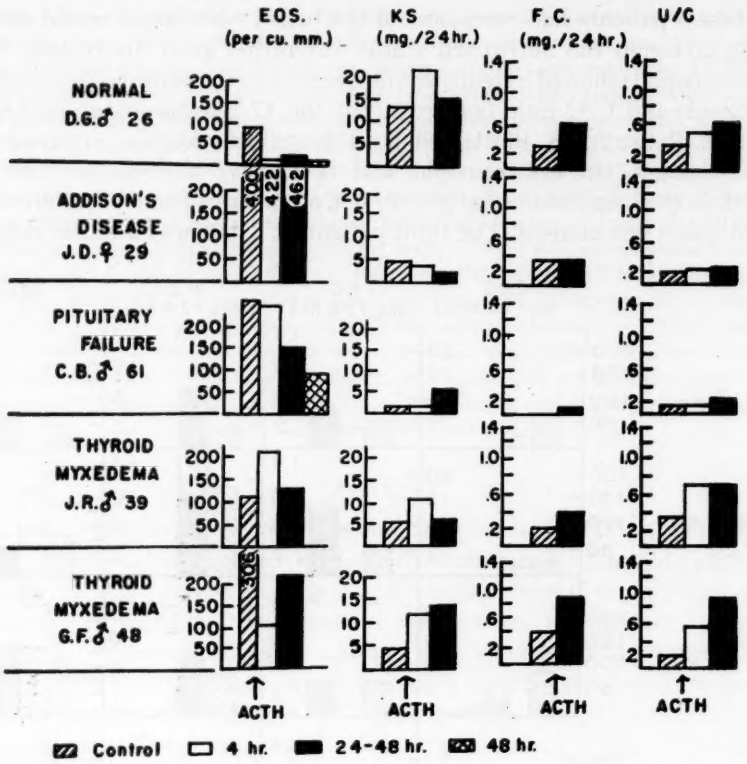

FIG. 2. ACTH response in adrenal, pituitary, and thyroid hypofunction compared with normal response.

U/C effect in forty-eight hours. Since an intact adrenal cortex is essential for the response to ACTH, it is obvious that patients with Addison's disease should not react in this way. Patient J.D. had an extremely high initial eosinophil count indicating no eosinopenic influences, and this count actually increased rather than decreased following administration of ACTH. The 17-ketosteroids, low initially, showed little change; formaldehydogenic corticoids and U/C similarly showed no change. A patient with pituitary failure theoretically has a nonfunctioning adrenal cortex only because the cortex is not being stimulated. This is illustrated in C.B. by a rather delayed type of reaction, showing a poor eosinophil and 17-ketosteroid response in four hours but a good response by the end of forty-eight hours. The formaldehydogenic corticoid and U/C changes were not as pronounced. It is important to mention here that had only a four-hour test been made,

the results would have been considered negative, whereas with the forty-eight-hour test the results were positive.

Recently it has been pointed out that the decreased metabolism associated with thyroid myxedema usually produces depression of adrenocortical function (8, 9). This was found to be partially true in 2 patients with thyroid myxedema (Fig. 2). The 17-ketosteroid levels initially were low for men of this age group. However, the responses to ACTH were fairly good, with the exception of the eosinophil effect in J.R. It appears, then, that what depression of adrenal function is present, is most probably due to depressed release of ACTH from the pituitary rather than decreased adrenal responsiveness.

In 9 patients with advanced carcinoma of the breast (Fig. 3) the averages of determinations showed poor 17-ketosteroid and U/C responses, although formaldehydogenic corticoid and eosinophil responses were satisfactory.

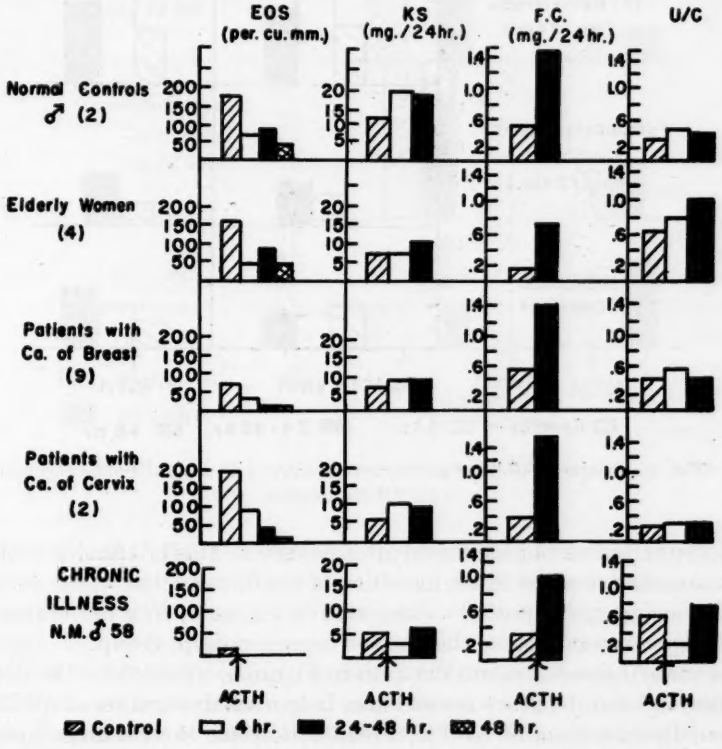

FIG. 3. Response to ACTH in patients with advanced cancer, debilitation, or aging. showing marked change in corticoids (F.C.) but little change in ketosteroids.

The 2 patients with carcinoma of the cervix showed essentially the same types of reaction, although the 17-ketosteroid and eosinophil changes were slightly greater than those in cancer of the breast. Whereas nutritional factors and effects of chronic debilitating disease undoubtedly play a part in reducing the adrenal response in patients with cancer, the factor of age is also important, as illustrated by 4 elderly women who were normal

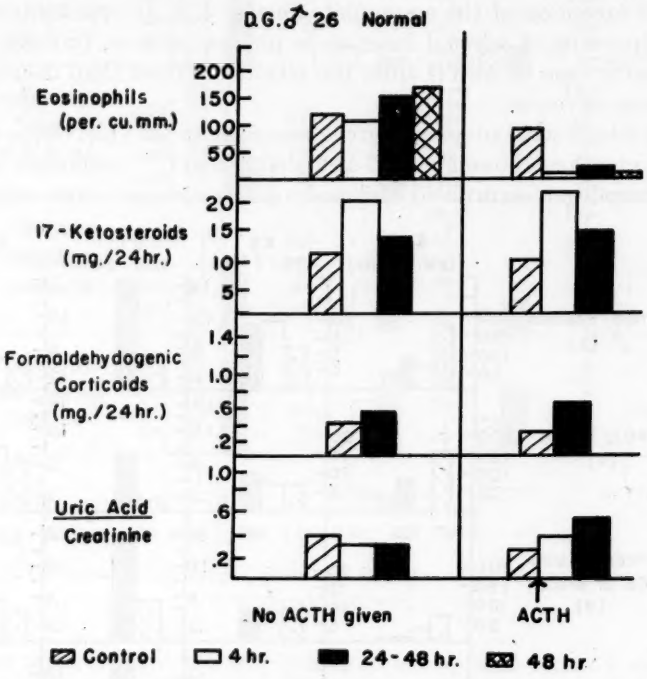

FIG. 4. Results of 48-hour test in normal male with and without exogenous ACTH stimulation.

with the exception of generalized arteriosclerosis. It is interesting that there was a marked increase in the excretion of the formaldehydogenic corticoids in all four groups of patients illustrated in Figure 3; on a percentage basis this response was greater than in the normal control group.

Of some interest also are the data in Figure 4, which show the determinations in an ambulatory normal man before administration of ACTH and after administration of ACTH, which took place several days later. The 17-ketosteroid and formaldehydogenic corticoid excretions were approximately the same in both situations, although there was slightly more

steroid excretion following administration of ACTH. The eosinophils showed a significant change only with ACTH, as did the U/C ratio. This does not mean that administration of ACTH is of no value as a diagnostic procedure, since adrenal response to either endogenous or exogenous ACTH is the objective; if found, the purpose of the test is fulfilled.

Figure 5 emphasizes the additional clinical application in a patient

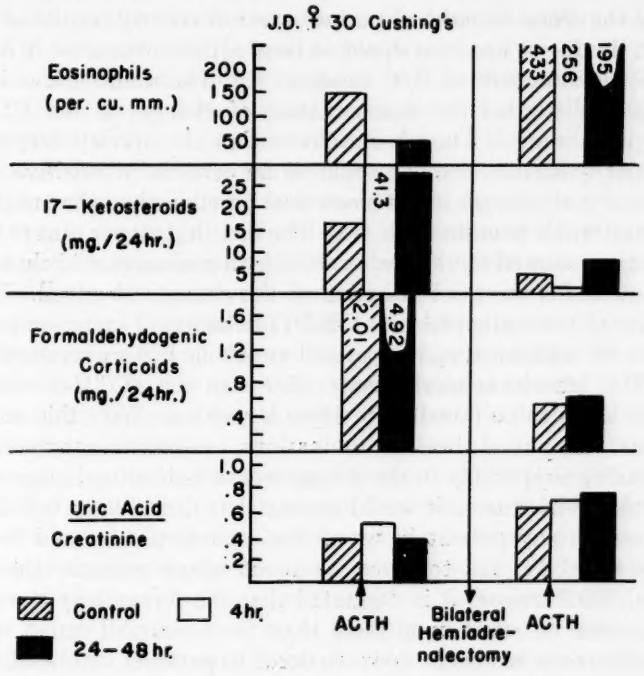

FIG. 5. Adrenal response to ACTH in Cushing's syndrome before and after surgical intervention.

(J.D.) who had Cushing's syndrome, due to bilateral adrenal hyperplasia. Prior to operation, the 17-ketosteroid and formaldehydogenic corticoid levels were elevated and showed tremendous increases after ACTH administration. The eosinophil response was significant, whereas the U/C was not. Three months following bilateral hemiadrenalectomy repetition of the test showed a good eosinophil response, a delayed 17-ketosteroid response, and absence of any of the other changes. This test was of considerable clinical value in evaluating the results of the surgical procedure performed on this patient.

DISCUSSION

Several interesting observations deserve further comment. Of the 24 over-all positive results obtained with ACTH stimulation tests, only 46 per cent showed significant U/C responses, whereas 79 to 92 per cent showed significant changes in the other determinations. It can be readily seen that the U/C changes were the least consistent and, therefore, the least reliable of the determinations. This was most probably due to the fact that the dietary intake of purines was not strictly regulated in these patients; this factor has been shown to be of prime importance in obtaining accurate measurements of U/C changes (10). Thorn and others have, for this reason, discarded the determination of changes in the U/C ratio. Equally interesting is Thorn's statement that the urinary determination of formaldehydogenic steroids would be an even more sensitive and specific indicator of changes in adrenocortical function than the measurement of 17-ketosteroids or eosinophils (11). The fact that 92 per cent of the positive reactions showed significant formaldehydogenic corticoid changes indicates that this is the most sensitive of the changes observed. The lower sensitivity of the eosinophil count and 17-ketosteroid excretion could well be due to the additional end-organ and metabolic factors involved, respectively. This introduces mechanisms other than the ACTH-adrenocortical axis, which has to be intact to produce a response. With this in mind, it is obvious, that any of these determinations, used alone, may occasionally be misleading—especially in the 4-hour test, which is used clinically more than is the 48-hour test. It would appear that the 48-hour test should be carried out in any patient in whom the clinical picture and the 4-hour response to ACTH fail to agree, or about whose response there is any question. Furthermore, it is suggested that the formaldehydogenic corticoid excretion be relied upon more than the eosinophil count, especially since eosinopenia has been shown to occur in patients with Addison's disease when given epinephrine or ACTH (2).

The 48-hour ACTH test is also of diagnostic value in patients with adrenal insufficiency secondary to anterior pituitary failure. Figure 2 shows a 4-hour response in the case of C.B., which was consistent with Addison's disease; however, with further stimulation, the adrenal did function and showed a good response in the 48-hour test. This was found to be true in other patients with pituitary insufficiency. Since the same type of reaction would be expected in anorexia nervosa (functional pituitary insufficiency) the ACTH test would be of no value in differentiation, which would have to rest on clinical, especially psychiatric, evidence (12).

The 2 patients (G.F. and J.R.) with thyroid myxedema (Fig. 2) seemed to show a secondary type of adrenal insufficiency, in that in each, the initial 17-ketosteroid excretion was lower than expected for that age group.

Both of these patients showed good responses, as measured with the 48-hour test. The question should be raised with regard to the diagnosis of thyroid myxedema as opposed to pituitary myxedema in these patients (8). G.F. was a 48-year-old man with myxedema of eighteen months' duration, whose blood cholesterol concentration was 412 mg. per 100 ml., and whose basal metabolic rates were −35 and −40 per cent on two occasions. He exhibited an excellent clinical response to thyroid administration and, chiefly for the latter reason, pituitary myxedema was deemed unlikely. Patient J.R. was a 39-year-old man with myxedema of ten years' duration. Laboratory determination revealed blood cholesterol levels of 685 and 826 mg. per 100 ml., respectively, a basal metabolic rate of −50 per cent and a pituitary gonadotropin assay of $>26<52.5$ m.u. These values probably indicate that the myxedema was primary and not secondary to pituitary failure. That the thyroid does not have to be functioning normally in order to obtain a good adrenal response to continued administration of ACTH is to be emphasized (9, 13). It is believed that because of thyroid myxedema in these patients, the decrease in 17-ketosteroid excretion was due to depression of the release of ACTH from the pituitary rather than to adrenal unresponsiveness; such a situation would account for the poor adrenal function. Whether or not this type of test can be used to differentiate pituitary myxedema from thyroid myxedema is doubtful, and must await further investigation.

The decreased adrenocortical responsiveness to ACTH in debilitated or aging patients, with or without cancer, has been noted by others (14). The effect of age in itself on adrenal response has been questioned (15). The factor of decreased protein intake, with or without increased utilization, is probably important and may result in decreased ACTH production, in a manner similar to that in which starvation decreases gonadotropin production. Heller and Shipley's (16) studies in aged subjects not given ACTH revealed a low 17-ketosteroid excretion, whereas the output of formaldehydogenic corticoids remained normal. They believed this possibly was due to a dual responsiveness of the adrenal to either a single or a dual ACTH production by the pituitary, which was affected by senescence. We used the same ACTH preparation in all our patients but the results in elderly and chronically ill patients were different from those obtained in normal subjects. The responses typically were characterized by marked formaldehydogenic corticoid increases, with only slight changes in the 17-ketosteroid excretion. This type of 17-ketosteroid response has been seen in debilitated individuals undergoing acute stresses (17). It seems, then, that in debilitated or aged individuals the adrenal loses its ability to produce 17-ketosteroid precursors, while maintaining its formaldehydogenic corticoid-producing capacity. The possibility of chronic stress

leading to adrenal exhaustion is not considered likely, in view of the significant increases in formaldehydogenic corticoid excretion.

It is worth emphasizing the study which was carried out on a normal man during ordinary activity with no exogenous ACTH stimulation as compared with the same situation when ACTH was given intramuscularly. As seen in Figure 4, the values differed only slightly. A value obtained when ACTH is given, then, may or may not be due to the exogenous ACTH alone. The important point seems to be that ACTH is specific in its action and a positive response therefore indicates that the adrenal is capable of increased secretion when stimulated. A negative response, on the other hand, is strong evidence for adrenal insufficiency.

However, when there is a significant positive reaction, it is difficult to state whether the adrenal has responded maximally, or only partially, to the administered ACTH. The patient with adrenal hyperplasia (Fig. 5) showed an excessive response but we could not say it was maximal, nor could we credit it entirely to the exogenous ACTH. Because of the influence of endogenous ACTH production, it would seem unlikely that an estimate of the quantity of adrenal hormone elaborated by a single fixed dose of ACTH could be accurate, unless the subject were protected from all outside influences and stresses which might lead to an increase in endogenous ACTH.

SUMMARY

In the course of the 48-hour ACTH test (intramuscular administration), the following determinations were made in 27 patients: urinary 17-ketosteroids, formaldehydogenic corticoids, uric acid/creatinine ratio, and total blood eosinophils. Of these, the formaldehydogenic corticoid value was the most sensitive index of adrenal responsiveness. Occasionally, when one of the determinations was indicative of a good response, the others showed no change. The U/C value rose in less than 50 per cent of the positive reactions obtained.

In panhypopituitarism, the result of the 4-hour ACTH test was negative, whereas the result of the 48-hour test was positive. In 2 patients with thyroid myxedema, the adrenal responsiveness was similar to that seen in pituitary failure, rather than in primary adrenal failure. In patients with chronic illness or advanced mammary cancer, there was a significant increase in urinary formaldehydogenic corticoids, but little, if any, in the 17-ketosteroids. This change in adrenal responsiveness was also seen in normal elderly women. Determinations in a normal man, with and without ACTH administration, suggested that the adrenal response is probably not due to the exogenous ACTH alone.

The 48-hour ACTH test has definite clinical usefulness and, when indicated, should be employed in determining the responsiveness of the adrenal cortex.

Acknowledgments

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EFFECT OF STRESS, TSH AND ACTH ON THE LEVEL OF HORMONAL I^{131} OF SERUM

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THAT rats subjected to a cold environment show histologic changes consistent with increased physiologic activity of the thyroid is well known. Nevertheless, the radioiodine (I^{131}) uptake of the gland tends to be low, as is also the case with the acute stress produced by formalin injection, trauma, tourniquet shock, anoxia, spinal cord transection, and x-irradiation (1–7). The level of the protein-bound I^{131} (PBI^{131}) of plasma likewise may be low (2, 4, 7).

Data derived from I^{131} uptake are difficult to evaluate in terms of actual secretory activity. In the present experiments a store of labelled thyroid hormone was first built up in the gland by prior administration of inorganic I^{131}. The activity of the butanol-soluble (thyroxine) fraction of serum was then determined daily during forty-eight hours before an episode of stress imposed by major surgery, and during a similar interval afterward. Under such circumstances an increased secretion of hormone would be attended by a rise in activity of the thyroxine component, provided the rate of systemic disposal of the hormone were not equally accelerated. In addition to a series of controls there were two other groups consisting of euthyroid subjects who were given TSH or ACTH in order to compare the effects of these hormones with those produced by stress. TSH is known to accelerate the rate of incorporation of I^{131} into the protein-bound iodine fraction of plasma (8, 9).

METHODS

Subjects were males ranging in age from 22 to 60 years. All were judged to be euthyroid, as indicated both by clinical evaluation and by the 24-hour thyroidal uptake of I^{131}. None of the surgical subjects was debilitated or critically ill prior to operation. There were no serious postoperative complications and ambulation was begun within twenty-four hours. Two days before the operation a dose of 100 microcuries of I^{131} was given orally. Blood samples, drawn at twenty-four and forty-eight hours thereafter, served to establish the preoperative level of activity of the thyroxine fraction. Samples taken at seventy-two and ninety-six hours after I^{131} administration corresponded to inter-

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vals of twenty-four and forty-eight hours after surgery. To serve as controls, blood samples were taken from 10 nonsurgical euthyroid subjects daily for four days after a similar dose of I^{131}.

An additional group of 9 nonsurgical subjects received an injection of 12 to 15 mg. of TSH[1] intramuscularly forty-eight hours after radioiodine had been administered. Some were given another dose of similar size at seventy-two hours. In addition to blood studies, daily readings were made over the thyroid gland in both the TSH and the control groups. The scintillation counter employed and the technique of measurement have previously been described (10).

A fourth group of 9 nonsurgical subjects received ACTH at forty-eight hours after the I^{131}, either as a single intravenous infusion of 25 mg. for six to eight hours, or by intramuscular injection (25 mg. four times daily) for the next forty-eight hours. The latter group was composed of patients with mild skin disorders or minimal asthma who were completely ambulatory, afebrile, and without signs of a current constitutional reaction. A sharp fall in the eosinophil count provided convincing evidence that a well marked physiologic response to the hormone had been produced.

The activity of serum was determined as follows: duplicate 1-ml. samples were extracted with 2 ml. of alkali-saturated butanol in the presence of NaOH, according to a recent simplification devised by Morton (11). The butanol extract was aspirated completely and placed in a Tracerlab 1-inch steel counting cup, the bottom of which had been covered with a circle of lens tissue to insure a uniform flat surface after drying. After the addition of 2 drops of 10 per cent "Tween 20" and 1 drop of 0.1 per cent silver nitrate, evaporation to dryness was accomplished under an infrared lamp and the cups counted under a shielded Geiger tube. The total count recorded was usually 4,000 and never less than 2,000. When the level of activity was extremely low, the counts were repeated in order to confirm the fact that detectable activity was actually present. Background counts ranged from 20 to 25 per minute. The activity of samples was usually more than 30 per cent above that of the background; however, a few were as low as 7 per cent. The accuracy of readings such as the latter, even with extended counting, is not of sufficiently high order to permit the detection of small changes; but, as noted later, no significance was attached to minor increases. Standards consisted of aliquots of the same solution which had been administered to the subject. The aliquot was evaporated to dryness in a cup, after being mixed with a butanol extract of human serum along with the reagents previously noted.

In the case of subjects who received TSH, and some of those who underwent surgery, PBI^{131} activity was also measured. Precipitation was achieved by the usual Somogyi zinc method for the removal of protein. After three washings with water the precipitate was dissolved in ammonia, washed into a counting cup, and after the addition of 1 drop of 0.1 per cent silver nitrate, dried in an oven at 60° to 80° C. Standards were added to a washed precipitate of human serum and processed in similar fashion.

RESULTS

The effects of surgery, TSH and ACTH, along with the behavior of control subjects are illustrated in Figure 1. In all four groups the 24-hour and 48-hour control values for the thyroxine fraction averaged approximately 3×10^{-5} per cent of the administered dose per ml. of serum. In

[1] The TSH was kindly supplied by Dr. George F. Kamen of the Armour Laboratories, Chicago.

the control series, in which serial observations were made for a total of ninety-six hours, the activity as plotted against time remained relatively constant throughout, with no consistent tendency either to rise or fall. Of the surgical patients, although 6 showed curves identical with those of the controls, the other 3 exhibited a well marked rise at twenty-four and forty-

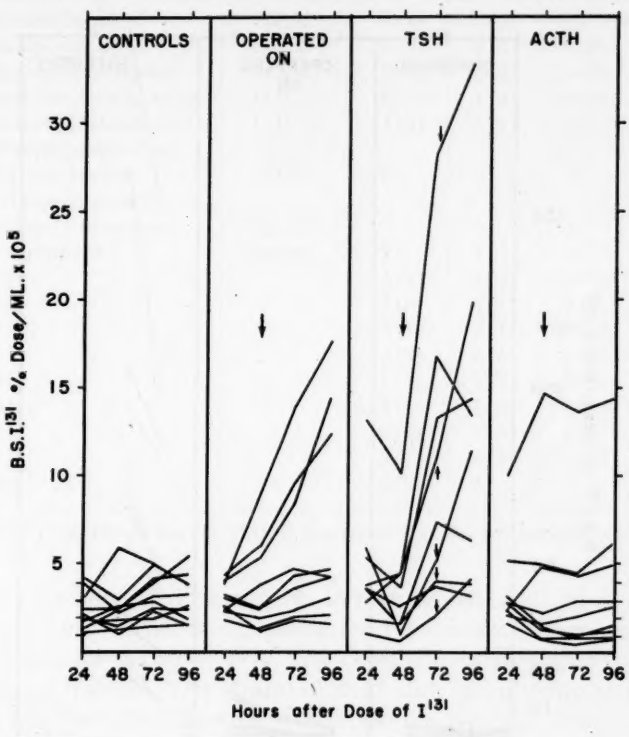

FIGURE 1

eight hours after surgery. Two of these underwent subtotal gastrectomy and the other a unilateral herniorrhaphy.

An effect from the TSH injection was discernible in 5 of the 9 patients receiving the hormone. Except for 1 instance in which initial control levels of activity were relatively high, the absolute rise, when it occurred, was similar to that encountered after surgery. With ACTH administration, the curves of activity remained flat and no instance of stimulatory effect was discernible. The topmost curve in this series was already at a relatively high level during the preliminary control period but there was no trend toward further elevation after ACTH was given.

The magnitude of hormonal I^{131} activity attained after stress may be compared with that encountered in hyperthyroidism, by reference to Figure 2. It is apparent that in most of the patients with the latter disorder, levels were attained at twenty-four and forty-eight hours which were well beyond the range observed in the postoperative patients. This does not necessarily prove that greater amounts of hormone are secreted in spon-

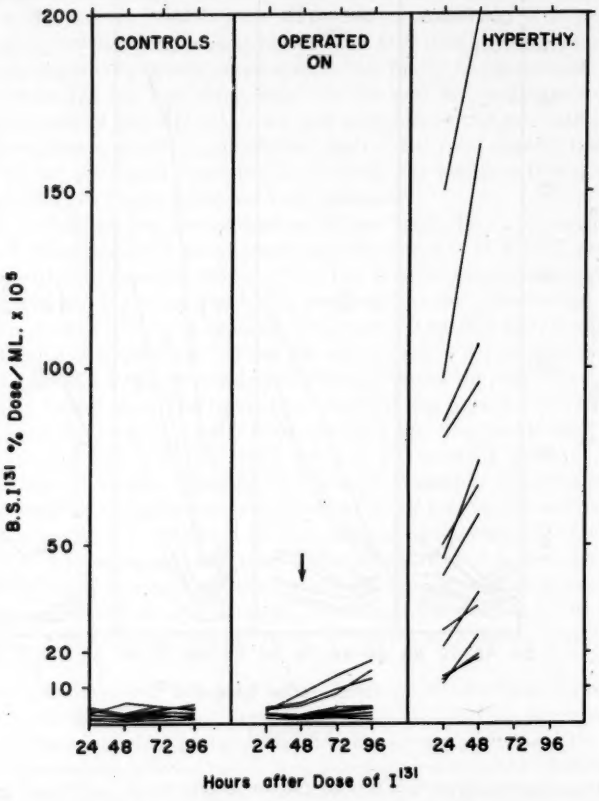

FIGURE 2

taneous hyperthyroidism. Since the store of hormone in the gland is low in this disease the secreted thyroxine should attain a higher specific activity of I^{131} than that arising from a normal gland. However the present data, derived solely from I^{131} activity of the blood, do not permit an estimate of the absolute output of thyroxine.

In Table 1 serial values of the butanol-soluble and protein-bound fractions are compared in those subjects who received TSH, and in some of

TABLE 1. COMPARISON OF BUTANOL-SOLUBLE AND PROTEIN-BOUND I^{131} FRACTIONS AFTER SURGERY OR TSH*

Patient No.	Procedure	Anesthesia	Time after I^{131} administration			
			24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	Herniorrhaphy	Spinal	4 (4)	6 (7)	10 (11)	12 (16)
2	Herniorrhaphy	Spinal	3 (2)	2 (3)	4 (6)	5 (5)
2	Hemorrhoidectomy	Sacral	3 (7)	2 (8)	4 (9)	5 (8)
4	Resection for Ca. colon	G.O.E.	2 (2)	1 (2)	2 (3)	2 (5)
5	Subtotal gastrectomy	G.O.E.	4 (4)	5 (7)	8 (10)	14 (17)
6	Subtotal gastrectomy	Spinal	4	—	14	17
7	Bilateral lumbar sympathectomy	G.O.E.	3	2	3	4
8	Subtotal gastrectomy	G.O.E.	2	2	2	3
9	Herniorrhaphy	Spinal	2	1	2	2
10	TSH		5 (2)	4 (4)	13 (15)	15 (18)
11	TSH		4 (3)	3 (3)	4 (5)	3 (4)
12	TSH		13 (15)	10 (14)	28 (38)	34 (50)
13	TSH		2 (2)	2 (3)	8 (9)	6 (8)
14	TSH		1 (1)	0.6 (2)	2 (3)	4 (7)
15	TSH		6 (6)	1 (4)	5 (7)	12 (13)
16	TSH		4 (9)	1 (5)	4 (8)	4 (7)
17	TSH		3 (4)	4 (5)	11 (15)	20 (29)
18	TSH		4 (2)	4	17	13

* Values in parentheses are for PBI^{131}; the remainder are for butanol-soluble I^{131}.

those who were subjected to surgery. It may be seen that when a rise in butanol activity occurred it was paralleled by a similar rise in the PBI^{131} fraction. Values for the latter were, in general, somewhat higher than those for the butanol fraction. This is most evident after seventy-two and ninety-six hours, whether the difference be expressed in terms of absolute values or on the basis of percentage. With the data at hand, it would be premature to speculate as to whether such a relationship might indicate a higher blood content of nonthyroxine organic I^{131} three or four days after the dose than during an earlier interval.

The thyroidal content of I^{131} as measured by serial external counts did not show a completely consistent response to TSH injection (Fig. 3). The general trend during the interval from twenty-four to forty-eight hours after the radioiodine and before TSH was given, was slightly upward, as would be expected for normal subjects. During the first twenty-four hours after TSH this rise was almost invariably reversed, so that the content of the gland became slightly lower. During the second twenty-four hours after TSH (seventy-two and ninety-six hours after I^{131}), the direction of

change was completely erratic. Other workers have not been able to demonstrate a clear-cut acceleration in the loss of I^{131} from the human thyroid when serial measurements were made by external counting following the administration of a single dose of TSH (9, 12). The cessation of decline or reversal observed forty-eight hours after the administration of TSH in the

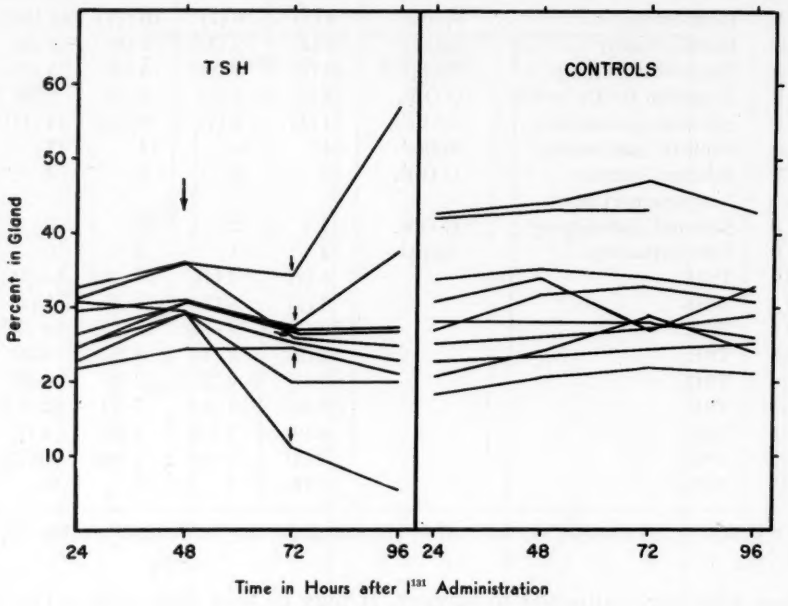

FIGURE 3

present studies might reflect an enhanced accumulation of inorganic I^{131} remaining in circulation from the original dose, or arising from degraded thyroxine.

DISCUSSION

The rise in the level of the hormonal I^{131} of serum in 3 of the patients who were subjected to surgery suggests that in these individuals thyroidal secretory activity was increased. Although a block in peripheral utilization of hormone could cause a similar change in the blood level, there is no reason to believe that such a phenomenon is ever associated with stress. Assuming that secretory activity was truly increased in these subjects, three questions arise: 1) Is the hypersecretion due to a primary stimulation of the gland, or is it a compensatory increase in activity resulting from a falling thyroxine level of the blood which, in turn, is secondary to an accentuated utilization or loss of hormone? 2) Why did 6 of the 9 pa-

tients fail to show a rise? 3) How can an increased secretory activity of the gland be reconciled with the well documented evidence that thyroidal uptake of I^{131} in the rat is depressed during stress (1–7)?

As to the first query, evidence is already available which indicates that the peripheral utilization of thyroid hormone in the rat is in fact increased during stress. Thyroidectomized rats maintained on thyroxine and exposed to cold exhibit a precipitous decline in the PBI level of the blood, whereas intact rats show little change (13). Likewise, rats exposed to cold while the thyroid gland is blocked with propylthiouracil require increased doses of thyroid hormone in order to stabilize the PBI level (14). If it be assumed that stress is accompanied by an exaggerated disposal of thyroxine, previous reports of low blood PBI^{131} activity in rats exposed to stressing conditions (2, 4, 7) can be explained on this basis. Furthermore the finding of unchanged levels of PBI in human patients subjected to surgery (15) would suggest that the accentuated disposal of hormone was in this instance compensated for by a corresponding increase in output by the thyroid. All of these findings are compatible with the concept that if the thyroid gland is stimulated by stress, the mechanism is of a compensatory nature.

In the present study the rise of hormonal I^{131} raises the question of whether there is a direct stimulation of the gland, independent of a secondary compensatory reaction in response to a falling hormone level of the blood. If the absolute level of serum thyroxine were known to have been elevated along with that of I^{131} activity, such a conclusion would be possible. Lacking chemical values for butanol-soluble iodine, one may only attempt deductions as to whether a rise in radioactivity represents an elevated hormone level. It may be reasoned that because control subjects showed a constant I^{131} activity of the blood after twenty-four hours, an equilibration had been attained between the specific activities of stored hormone in the gland and that in the blood. In such a case a rise in the patients who were operated on would indicate an absolute rise in the level of blood hormone. However, in spite of the present indication of rapid equilibration, there is evidence that blood thyroxine is replaced at a rate of only 30 per cent per day (16). It may therefore, be questioned whether complete equilibrium is possible, even by forty-eight hours. Nevertheless it is fair to conclude that the present results are sufficiently suggestive of a direct thyroid stimulation to warrant further investigation.

The failure of 6 patients of the surgical series to show a rise of I^{131} activity might be explained on the basis of individual variations in responsiveness to a stimulus of near-threshold magnitude. Another possibility is that an increased output of hormone actually did occur but was exactly balanced by an accelerated peripheral disposal.

The matter of thyroidal uptake of I^{131} being depressed in rats subjected to stress, despite other evidence which points toward thyroid stimulation, deserves brief comment. It is possible that the rat is unique among animals in that a stimulus mediated by TSH is manifested by an unusually rapid synthesis and release of hormone, so that the gland content does not rise. A more likely explanation concerns the effect of the adrenocortical hormone, which is known to increase during stress. It is well established that cortisone interferes with the uptake of I^{131} by the thyroid. Whether the mechanism involves a direct thyroidal effect (17) due perhaps to an antagonism of TSH at the target-organ level (18, 19), or is related to a low plasma iodide concentration resulting from an enhanced renal excretion of this ion (15, 20), is uncertain. ACTH does not appear to retard the *release* of I^{131} from the gland of intact rats (21, 22). Therefore, during stress, it is possible that hypersecretion may occur in the face of decreased uptake by the gland.

SUMMARY

The stress of major surgery was accompanied by a rise in the level of the hormonal I^{131} of serum in 3 of 9 patients. A similar effect was produced by the injection of small doses of TSH in 5 of 9 normal subjects. ACTH did not influence the level.

Counts made over the thyroid gland after the injection of TSH indicated a slight loss of activity during the first twenty-four hours, but in the second twenty-four hours there was more often a secondary rise or no further decline.

The results are compatible with the concept that acute stress causes a release of thyroid hormone from the gland. Experiments reported by others indicate that an increased utilization of hormone by peripheral tissues also occurs. The present studies suggest, but do not conclusively prove, that the intensified thyroid activity is not solely a secondary response promoted by a declining blood thyroxine level, but may include an independent primary stimulation of the gland.

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IN VIVO METABOLISM OF NEUTRAL STEROID HORMONES*

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FOR almost twenty years workers in the steroid field have had the objective of relating the tissue steroid hormones to specific urinary metabolites. To this end, studies on the metabolism of steroid hormones have been made by the following approaches: a) a search for steroid hormones from testes, adrenals, placentae and ovaries, b) a search for steroid compounds in urines from animals and human subjects, and c) a search for steroid hormone metabolites in urine after the administration of steroid hormones to human subjects. As a result of these studies, almost 100 naturally occurring steroids have been isolated from tissue and urinary sources and 7 neutral steroid hormones have been submitted to *in vivo* metabolic investigations. On the basis of new experimental data, particularly with respect to the metabolism of adrenocortical hormones and in the light of some of the earlier findings, it is now possible to make some tentative generalizations which aid in the understanding of the basic relationship between the tissue steroid hormones and their urinary metabolites in human subjects. These generalizations may also be important in understanding the changes in steroid metabolism that occur in such conditions as old age, stress, and disease.

It is the purpose of this communication to indicate the general statements that may be made concerning the *in vivo* metabolism of the neutral steroid hormones. Of particular interest is a new generalization concerning the relationship between chemical substitution of the steroid nucleus and the reduction of the Δ^4-3-ketone in ring A of the neutral steroids. On the basis of this generalization, two further points may be discussed, namely, the origin of the 17-ketosteroids and the mechanism of the *in vivo* conversion of C_{21} steroids to 17-ketosteroids.

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Reduction of Δ^4-3-ketone in C_{19} steroids

Table 1 lists the *in vivo* metabolism of four C_{19} compounds, three of which possess the Δ^4-3-ketone grouping in ring A. The fourth compound, dehydroepiandrosterone, possesses the Δ^5-3β-hydroxy grouping in rings A and B and will be discussed separately.

TABLE 1. THE *in vivo* METABOLISM OF C_{19} STEROIDS IN HUMAN SUBJECTS

Steroid admin'd. (mg. per day)	Reference	Type of subject (route of admin.)	C_{19} steroids isolated from urine (% of admin'd. dose)		Ratio $\frac{5\beta}{5\alpha}$
			5β	5α	
Testosterone (171)	(1)	Normal women (I.M.)	Etiocholane-3α-ol-17-one (10.1)	Androsterone (18)	0.56
Testosterone (90)	(2)	Normal men (I.M.)	Etiocholane-3α-ol-17-one (17)	Androsterone (21)	0.81
Testosterone (100)	(3)	Normal men (I.M.)	Etiocholane-3α-ol-17-one (25.7)	Androsterone (10.3)	2.50
Δ^4-Androstene-3,17-dione (350)	(4)	Hypogonadism; male (I.M.)	Etiocholane-3α-ol-17-one (16.1)	Androsterone (15.7)	1.03
Δ^4-Androstene-3,17-dione (100)	(3)	Normal men (I.M.)	Etiocholane-3α-ol-17-one (21.9)	Androsterone (21.1)	1.04
Δ^4-Androstene-3,17-dione (500)	(5)	Rheumatoid arthritis (I.M.)	Etiocholane-3α-ol-17-one (523 mg.)*	Androsterone (515 mg.)*	1.02
Dehydroepiandrosterone (68) (mean)	(6)	Hypopituitarism; male (I.M.)	Etiocholane-3α-ol-17-one (6.7)	Androsterone (12)	0.56
		Addison's disease; male (I.M.)	Etiocholane-3α-ol-17-one (10.1)	Androsterone (2.2)	4.6
		Addison's disease; female (I.M.)	Etiocholane-3α-ol-17-one (3.3)	Androsterone (5.7)	0.58
Dehydroepiandrosterone	(7)	Normal women (I.M.)	Etiocholane-3α-ol-17-one (50 mg.)*	Androsterone (20 mg.)*	2.5
		Addison's disease; male (oral)	Etiocholane-3α-ol-17-one (28)	Androsterone (30)	0.6
Andrenosterone (100)	(9)	Rheumatoid arthritis; male (oral)	Etiocholane-3α-11β-diol-17-one (0.8)	11β-Hydroxyandrosterone (5.7)	0.19
			Etiocholane-3α-ol-11,17-dione (0.4)	11-Ketoandrosterone (0.7)	

* Amount isolated from an aliquot.

It is clear from Table 1 that both testosterone and Δ^4-androstene-3,17-dione are metabolized to androsterone and etiocholane-3α-ol-17-one (1–5). These two substances differ in their stereoisomeric relationship between rings A and B and more specifically, the spatial position of the hydrogen atom at Carbon 5. In androsterone the hydrogen atom at Carbon 5 is

considered to be 5α and is so designated in the tables. In etiocholane-5α-ol-17-one, the hydrogen atom at Carbon 5 is in the 5β position.

In 3 *in vivo* metabolism experiments involving Δ^4-androstene-3,17-dione (3–5) at dosage levels varying from 100 to 500 mg. per day, the metabolites androsterone and etiocholane-3α-ol-17-one were excreted in roughly equal amounts so that the ratios of the 5β-isomer to 5α-isomer were 1.03, 1.04 and 1.05, respectively.

When testosterone was administered in doses varying from 90 to 171 mg. per day, the principal metabolites were androsterone and etiocholane-3α-ol-17-one (1–3). The ratio of 5β to 5α isomer varied from 0.56 to 2.50 (Table 1). The mean ratio for the 3 experiments was 1.29.

Five experiments with dehydroepiandrosterone have been reported (6–7). The ratio of etiocholane-3α-ol-17-one (5β isomer) to androsterone (5α isomer) varied from 0.56 to 4.6. The mean ratio was 1.70. It is to be remembered that all the experiments in this group have been performed only on a semiquantitative basis, and considerable variation is to be expected. Further, in the metabolism of dehydroepiandrosterone it may be expected that the first metabolic step would be the formation of Δ^4-androstene-3,17-dione by oxidation of the 3β-hydroxy group. That this can take place in steroid-producing tissue has been demonstrated (8).

Of particular interest is the fact that for three C_{19} steroids without oxygen at Carbon 11, the reduction of the Δ^4-double bond *in vivo* proceeded with the formation of both the 5β and 5α stereoisomers essentially in equal proportions. In studies on the *in vivo* metabolism of adrenosterone (9) which possesses a ketone group at Carbon 11, a difference in ratio of 5β to 5α stereoisomers was found. The bulk of the reduced 17-ketosteroids formed and excreted in the urine have the 5α configuration. Actually the ratio of 5β to 5α isomers was found to be 0.19. This ratio appears to be significantly lower than the mean ratios of 1.29, 1.03 and 1.70 found for testosterone, Δ^4-androstene-3,17-dione, and dehydroepiandrosterone, respectively. It appears therefore that the oxygen substitution at Carbon 11 orients the reduction at Carbons 4 and 5 primarily to the 5α configuration. Two additional points in metabolism have been learned from these studies. First, that the oxygen function at Carbon 11 is not removed in metabolism, that is, no 11-desoxy-C_{19}-steroids were formed. Secondly, the 11-keto group of adrenosterone was reduced in part to the 11β-hydroxy group. Actually, by far the greatest concentration of metabolites contained the 11β-hydroxy grouping (9).

Metabolism of C_{21} steroids to C_{21} metabolites

Six C_{21} steroids possessing the Δ^4-3-ketone grouping in ring A, of which 5 were biologically active, naturally-occurring steroids, have been studied

by the *in vivo* technic in human subjects (10–17). Both C_{21} metabolites and C_{19} metabolites have been isolated from C_{21} steroids.

Table 2 lists the C_{21} steroid metabolites derived from the C_{21}-Δ^4-3-ketones. The most striking single point in the metabolism of these compounds is the presence of reduction products in the pregnane or 5β series. Progesterone is the only member of this group in which metabolites of the 5α series have been isolated (14–16), and even in this instance the amount

TABLE 2. THE *in vivo* METABOLISM OF C_{21} STEROIDS TO C_{21} METABOLITES IN HUMAN SUBJECTS

Steroid admin'd (reference)	Dose (mg. per day)	Type of subject (route of admin.)	C_{21} steroids isolated from urine (% of admin'd dose)		Ratio $= \frac{5\beta}{5\alpha}$
			5β	5α	
Cortisone (10)	500	Normal man (oral)	Tetrahydro F (3α-ol) (0.11) Tetrahydro E (3α-ol) (2.5)	Not isolated	$\frac{2.6}{0}$
Hydrocortisone (11)	500	Scleroderma; male (oral)	Tetrahydro E, F (3α-ol) (1.4)	Not isolated	$\frac{1.4}{0}$
21-Desoxycortisone (12)	100–200	Rheumatoid arthritis; women (oral)	Pregnane-3α, 17α-diol-11, 20-dione (0.4)	Not isolated	$\frac{0.4}{0}$
Desoxycorticosterone (13)	116	Addison's disease; female (oral)	Pregnane-3α, 20α-diol (1.4)	Not isolated	$\frac{1.4}{0}$
	103	Addison's disease; male (oral)	Pregnane-3α, 20α-diol (2.4)	Not isolated	$\frac{2.4}{0}$
	119	Addison's disease; male (oral)	Pregnane-3α, 20α-diol (2.7)	Not isolated	$\frac{2.7}{0}$
	102	Hypogonadism; male (oral)	Pregnane-3α, 20α-diol (3.1)	Not isolated	$\frac{3.1}{0}$
Progesterone (14)	208 (mean)	Rheumatoid arthritis; female (I.M.)	Pregnane-3α, 20α-diol (6.0) Pregnane-3α-ol-20-one (0.6)	Allopregnane-3α, 20α-diol (0.4)	16.5
Progesterone (15)	100	Normal male (I.M.)	Pregnane-3α-ol-20-one (3) Pregnane-3α, 20α-diol(?)	Allopregnane-3α-ol-20-one (0.4) Allopregnane-3α, 20α-diol(?)	7.5
Progesterone (16)	50	Postmenopausal women (I.M.)	Pregnane-3α,20α-diol (63)	Allopregnane-3α,20α-diol (1.9)	33
	100	Rheumatoid arthritis; male (I.M.)	Pregnane-3α, 20α-diol (8.6)	Allopregnane-3α, 20α-diol (0.3)	27
11-Dehydrocorticosterone (17)	100 (mean)	Addison's disease	Pregnane-3α,-20α-diol-11-one (4.3)		$\frac{4.3}{0}$

of 5α stereoisomer found has been a small fraction of the concentration of 5β form. Studies with cortisone (10), hydrocortisone (11), 21-desoxycortisone (12), desoxycorticosterone (13), and 11-dehydrocorticosterone (17) have yielded only the 5β stereoisomers. Thus it may be concluded that the ratio of 5β to 5α is 7.5 or greater, and further that the presence of the side-chain at Carbon 17 directs the reduction of the double bond of the Δ^4-3-ketone in ring A primarily to the 5β configuration, whether or not an oxygen function is present at Carbon 11.

Metabolism of C_{21} steroids to 17-ketosteroids

C_{21} steroids possessing oxygen functions at Carbons 17 and 20 may be metabolized to 17-ketosteroids, as indicated in Table 3. The metabolism of cortisone, hydrocortisone and 21-desoxycortisone to etiocholane-3α, 11β-

TABLE 3. THE *in vivo* METABOLISM OF C_{21} STEROIDS TO C_{19} METABOLITES IN HUMAN SUBJECTS

Steroid adminis'd (mg. per day)	Reference	Type of subject (route of admin.)	C_{19} steroids isolated from urine (% of admin'd dose)		Ratio $\frac{5\beta}{5\alpha}$
			5β	5α	
Cortisone (500)	(10)	Normal man (oral)	Etiocholane-3α,11β-diol-17-one (0.98) Etiocholane-3α-ol-11,17-dione (0.5)	11β-Hydroxyandrosterone (0.24)*	6.0
Hydrocortisone (500)	(11)	Scleroderma; male (oral)	Etiocholane-3α,11β-diol-17-one (1.75) Etiocholane-3α-ol-11,17-dione (0.69)	11β-Hydroxyandrosterone (0.1)*	24.4
21-Desoxycortisone (100–200)	(12)	Rheumatoid arthritis; women (oral)	Etiocholane-3α,11β-diol-17-one (0.24) Etiocholane-3α-ol-11,17-dione (0.27)	11β-Hydroxyandrosterone (0.17)*	4.1

* Uncertainty exists as to the amount of material being metabolized from the respective steroids. At least a portion may arise endogenously.

diol-17-one and etiocholane-3α-ol-11,17-dione has been definitely demonstrated (10–12). The metabolism of the C_{21} steroids to 11β-hydroxyandrosterone has been indicated, but the quantities isolated were so small as to be possibly confused with the endogenous production. However, even if the quantity of the 5α isomer isolated is assumed to be a true metabolite, then the ratio of 5β to 5α 17-ketosteroids varied from 4.1 to 24.4 for the three C_{21} steroids.

Mechanism of in vivo *conversion of C_{21} steroids to 17-ketosteroids*

In a preceding section, it was demonstrated that adrenosterone (C_{19} steroid with C-11-oxygen) was metabolized primarily to 5α metabolites

(11β-hydroxyandrosterone and 11-ketoandrosterone) and that the ratio of 5β to 5α isomers was 0.19. It was further demonstrated that cortisone, hydrocortisone and 21-desoxycortisone were metabolized primarily to 5β 17-ketosteroids. This is illustrated by the fact that the ratio of 5β to 5α 17-ketosteroid metabolites is 4.1 or greater. Therefore, it is likely that the principal pathway for the sidechain removal of a steroid such as cortisone, is after the double bond in ring A is reduced.

It is postulated, on the basis of available evidence, that a steroid such as cortisone is reduced primarily to the dihydro (or tetrahydro) pregnane (5β) derivative. Only after this nuclear reduction in ring A is accomplished, is the sidechain removed by oxidative means forming the 17-ketosteroid.

The alternative, *i.e.*, the oxidative removal of the sidechain before reduction in ring A, is untenable as the principal pathway, since if this were the case the first product formed would be either adrenosterone in the case of cortisone or 11-hydroxy-Δ^4-androstene-3,17-dione in the case of hydrocortisone. These compounds in turn, as has already been demonstrated, would yield predominantly the 11-oxygenated 17-ketosteroids in the androstane series (5α); but this is contrary to fact. Actually, both cortisone and hydrocortisone yield predominantly 5β stereoisomers. It is therefore concluded that the bulk of C_{21} compounds which are converted to C_{19} 17-ketosteroids must first undergo reduction in ring A and secondly, sidechain splitting.

Predicted relationship between adrenocortical steroids and 17-ketosteroids

17-Ketosteroids: On the basis of evidence already reviewed, we can arrange the interrelationship between certain steroids in the adrenal cortex and the urinary 17-ketosteroids. Androsterone arises primarily as a result of the metabolism of Δ^4-androstene-3,17-dione and dehydroepiandrosterone. Preliminary evidence indicates that 17-hydroxyprogesterone and 17-hydroxy-11-desoxycorticosterone may contribute small amounts of androsterone. Etiocholanolone-3α-ol-17-one would arise from three sources. These sources include Δ^4-androstene-3,17-dione and dehydroepiandrosterone, in about the same magnitude as the formation of androsterone. Etiocholane-3α-ol-17-one would also result from the metabolism of such compounds as 17-hydroxy-11-desoxycorticosterone and 17-hydroxyprogesterone.

We may consider the 11-oxygenated 17-ketosteroids as the sum total of the 11-keto and 11-hydroxy derivatives. This is a reasonable procedure, since the 11-keto and 11β-hydroxy groups are interchangeable in metabolism. The 11-oxygenated androsterone derivatives arise practically exclusively from 11-oxygenated Δ^4-3-keto-17-ketosteroids; in other words, the

11-oxygenated androsterone derivatives would arise from adrenosterone and perhaps 11β-hydroxy Δ^4-androstene-3,17-dione. These two 11-oxygenated α,β-unsaturated ketones would also contribute some 11-oxygenated etiocholane derivatives. However, the bulk of the 11-oxygenated etiocholane derivatives would arise as a result of the metabolism of such substances as hydrocortisone and cortisone.

SUMMARY AND CONCLUSIONS

On the basis of the experimental facts now in hand, the following tentative generalizations may be made concerning the *in vivo* metabolism of neutral steroid hormones:

1. C_{19} steroids possessing the Δ^4-3-ketone grouping in ring A and an additional oxygen atom at Carbon 17 are reduced at Carbons 4 and 5, with the formation of approximately equal amounts of the 5α and 5β stereoisomers.

2. C_{19} steroids possessing the Δ^4-3-ketone grouping in ring A and additional oxygen atoms at Carbons 11 and 17 are reduced at Carbons 4 and 5 with the formation of both 5β and 5α stereoisomers, the latter in significantly greater amounts.

3. C_{21} steroids possessing the Δ^4-3-ketone grouping in ring A, irrespective of the presence or absence of oxygen at Carbons 11, 17 and 21, are reduced at Carbons 4 and 5 with the formation of the 5β stereoisomers and only minute amounts of the 5α stereoisomer, if at all.

4. C_{21} steroids possessing the Δ^4-3-ketone grouping in ring A and oxygen at Carbons 17 and 20 are converted in part to C_{19} saturated 17-ketosteroids. The conversion of these C_{21} steroids to the C_{19} saturated 17-ketosteroids proceeds primarily by way of a saturated C_{21} intermediate.

These generalizations are tentative until more *in vivo* data are available on the influence of mode of steroid administration, age, sex, stress and clinical state of the individual.

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OBSERVATIONS ON THE CALCULATION OF THYROID WEIGHT, USING EMPIRICAL FORMULAE

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THE importance of knowledge of the weight of the thyroid gland in the estimation of the amount of radioactive iodine to be given for therapy of hyperthyroidism has resulted in attempts to develop objective methods for weight evaluation. In this hospital the thyroid gland has been outlined according to previously reported methods (1, 2, 3), using a motor-driven directional scintillation counter. From the resultant outline, variously named gammagram or scintogram, the weight of the thyroid has been calculated. This method of calculation has been reported (1) to be accurate within 10 per cent. The weight calculation is made according to the empirical equation:

$$\text{Weight of each lobe} = \text{Maximum height in cm.} \times \text{Surface area in cm.}^2 \times K \quad \text{(I)}$$

K is a constant supposedly relating the height of a lobe to its third dimensional projection, taking into account the density of thyroid tissue. It is assumed: a) the height is proportional to the size of the gland; b) the surface area (in the anterior projection) is proportional to the size of the gland; or c) their product is proportional to the size of the gland. These assumptions have been reported to have been verified and the K factor calculated from study of 10 patients whose thyroid glands were removed at operation or necropsy soon after the gammagrams or scintograms had been made.

If the weight of the thyroid gland could be calculated with an accuracy of 10 per cent, this would represent a considerable improvement in technic for the therapeutic use of radioiodine, since the estimation of thyroid weight on the basis of physical examination is known to be fraught with error greater than 10 per cent.

To check the accuracy of this method of weight calculation and to test the assumptions upon which it is based, the following studies were carried out.

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METHODS

Fifteen patients whose thyroid glands were outlined according to the aforementioned method were chosen for study. Thirteen of these patients had thyroidectomies for hyperthyroidism. There were 2 patients without thyroid disease, in whom autopsies were performed. No patient with nodules in the thyroid was included. The thyroid outline made at a time nearest to the time of surgery (a few days to two or three weeks before) or necropsy (a few days before) was used in all cases. In approximately one half of the cases the measurements of the gammagrams used were the mean measurements made by three members of the laboratory. (These had been obtained about one year previously, during studies of the reproducibility and variability of thyroid outlines (3).) The measurements of the fresh, unfixed thyroid tissue removed at operation or necropsy were checked by the author, a member of the Pathology Department, and one of the technicians in the laboratory, in most cases. Correction of the weight of thyroid tissue had been made prior to calculation of the percentage error for the amount of tissue left in the neck after subtotal thyroidectomy. The surgeon's estimate of tissue remaining after subtotal thyroidectomy, as performed, was 5 to 10 grams at most.

Sixteen additional cases were studied at necropsy by Mr. Benson of this laboratory. Through the kindness of Dr. Bela Halpert, Chief of the Laboratory Service, the trachea, larynx, and thyroid gland were removed in one portion and saved for examination. No radioactive iodine studies had been made on these patients, who died of diseases unrelated to the thyroid. The strap muscles were trimmed away and anterior *visual* outlines of the thyroid made. From these outlines the maximum height and surface area were measured. The weight of each lobe was then calculated according to equation (I). Most of these glands were studied immediately after removal from the body. A few were frozen until time permitted measurement, and a few were examined after the tissues had been fixed in formalin. These variations in procedure did not alter the results of calculation of the percentage error.

RESULTS

Table 1 summarizes the data on the 15 cases studied before operation or necropsy, by means of the gammagraphic technic. Inspection of this table reveals marked variation between the calculated and actual weights of each lobe and of the whole gland. The calculated weights are always larger than the actual weights, extremes being 6.2 to 147.6 per cent, with a mean positive error of 49 per cent.

TABLE 1. THYROID MEASUREMENTS FROM GAMMAGRAMS; OPERATIVE AND NECROPSY MATERIAL

No.	Patient	Right lobe					Left lobe					Calc. total wt. (Gm.)	Actual total wt. (Gm.)	Error %
		Gammagram			Surg. or autopsy spec.		Gammagram			Surg. or autopsy spec.				
		Ht. (cm.)	Surf. area (cm^2)	Calc. wt. (Gm.)	Actual ht. (cm.)	Actual wt. (Gm.)	Ht. (cm.)	Surf. area (cm^2)	Calc. wt. (Gm.)	Actual ht. (cm.)	Actual wt. (Gm.)			
1	I.M.W.	8.9	23.6	67.8	7.3	35.1	—	—	—	—	—	67.8	35.1	93.2
2	J.T.	5.3	12.3	17.98	4.7	14.0	—	—	—	—	—	17.98	14.0	28.6
3	L.S.	6.15	13.32	26.48	6.2	21.0	5.5	16.23	34.85	5.5	28.0	61.33	49.0	25.2
4	T.R.H.	8.4	24.9	67.56	6.0	25.0	7.5	22.2	53.78	6.0	24.0	121.34	49.0	147.6
5	I.C.	5.2	10.4	17.47	—	—	4.9	10.55	16.69	—	—	34.16	25.0	36.6
6*	G.S.	5.7	11.47	21.12	5.6	11.8	5.24	9.03	15.28	4.3	10.0	36.47	21.8	67.0
7	W.S.	5.2	10.2	17.13	4.0	14.1	5.6	11.5	20.80	5.2	14.5	37.93	28.6	32.6
8	C.P.	9.2	33.75	100.3	9.1	80.0	9.5	32.3	99.1	9.5	69.4	199.4	159.4	25.1
9	S.B.	8.5	31.7	87.0	—	54.3	9.7	38.6	120.94	8.8	71.3	207.94	135.6	53.3
10	W.D.	7.3	17.9	42.2	6.5	—	6.1	14.1	27.78	5.6	—	69.98	65.9	6.2
11	S.H.	6.6	17.4	37.09	5.9	—	6.0	15.4	29.84	5.6	—	66.93	32.3	107.2
12	J.H.	9.4	31.4	95.34	7.8	74.7	9.2	24.3	72.21	8.7	67.2	167.55	146.9	14.1
13	G.P.	5.67	13.73	25.14	5.7	24.45	4.93	11.7	18.63	5.0	16.40	43.77	40.85	7.1
14	A.N.	7.5	18.1	43.9	—	30.0	7.5	15.6	37.8	—	37.0	81.7	67.0	21.9
15*	J.M.	5.5	10.5	18.6	5.4	9.4	5.0	8.2	13.2	5.1	9.2	31.8	18.7	70.0
													Mean error	49%+

* Necropsy data.

In Table 2 are presented similar data obtained from the 16 necropsy specimens. Also included in this table are the recalculated K factors based on the actual measurements and weights. It should be emphasized that the anterior *visual* outline is smaller than the anterior gammagram because the eye cannot see through the trachea, whereas the scintillation counter will detect gamma rays coming from either the anterior or posterior portions of the gland. Gamma rays from either source will result in deflections in the completed gammagram which are apparently anterior. There will be more distortion of the outline from those gamma rays coming from the posterior portion of the gland, since it is farther from the crystal of the counter. Inspection of Table 2 likewise reveals marked discrepancies between the calculated and the observed weights. Differing from the data obtained by the gammagraphic technic, the variations may be either positive or negative, extremes being +74 per cent to −38.9 per cent error, with a mean error of +9.7 per cent. These variations are likewise reflected in the recalculated K values shown in this table.

To test the assumptions made in equation (I), analyses of the data were carried out to answer the following questions:

a. Does the height of the thyroid lobe on the gammagram or outline vary with the actual weight of the lobes removed at surgery or necropsy?

In Figure 1 the height (maximum) of each lobe on the gammagram is

TABLE 2. THYROID MEASUREMENTS FROM VISUAL OUTLINES; NECROPSY MATERIAL

Necropsy No.	Right lobe				Left lobe				Calc. total wt. (Gm.)	Actual total wt. (Gm.)	Error %	K recalc. (rt. lobe)	K recalc. (lt. lobe)	Mean recalc. K (both lobes)
	Visual outline		Calc. wt. (Gm.)	Actual wt. (Gm.)	Visual outline		Calc. wt. (Gm.)	Actual wt. (Gm.)						
	Ht. (cm.)	Surf. area ($cm.^2$)			Ht. (cm.)	Surf. area ($cm.^2$)								
1	4.8	8.4	13.0	10.6	4.4	7.1	10.1	9.2	23.1	19.8	16.7+	.263	.294	.279
2	4.2	5.2	7.05	—	4.2	5.9	8.0	—	15.05	14.3	7.3+	—	—	.307
3	4.7	4.2	6.38	6.0	3.55	3.4	3.90	5.4	10.28	11.4	9.8−	.304	.447	.376
4	5.0	7.3	11.79	6.8	3.9	3.1	3.91	4.3	15.70	11.1	41.4+	.186	.356	.271
5	4.2	5.2	7.05	7.8	3.7	5.9	6.5	6.5	13.88	14.3	2.9−	.357	.298	.328
6	3.9	—	—	—	3.1	—	—	—	8.25	13.5	38.9−	—	—	.528
7	3.5	4.5	5.09	5.9	4.0	3.7	4.0	5.1	9.09	11.0	17.4−	.375	.411	.393
8	5.0	6.1	9.85	5.5	4.7	5.7	8.65	5.7	18.50	11.2	65.2+	.180	.213	.197
9	4.4	8.45	12.01	6.3	4.4	6.85	9.74	6.2	21.75	12.5	74.0+	.169	.199	.184
10	4.9	8.6	13.6	14.7	4.4	7.6	10.8	12.3	24.4	27.0	9.6−	.349	.368	.359
11	4.3	7.6	10.56	11.1	4.35	8.2	11.52	12.0	22.08	23.1	4.4−	.340	.336	.338
12	3.95	6.5	8.39	7.0	3.35	5.15	5.57	5.7	13.96	12.7	9.9+	.273	.330	.302
13	4.2	6.6	8.95	7.9	4.8	8.15	12.64	11.2	21.59	19.1	11.3+	.285	.286	.285
14	3.0	4.85	4.70	—	3.5	5.3	5.99	—	10.69	10.7	0	—	—	.323
15	4.1	7.7	10.2	—	5.0	7.7	12.44	—	22.64	27.1	16.4−	—	—	.387
16	—	—	—	—	3.9	5.8	7.31	5.7	—	—	28.2	—	.252	—
													Mean error 9.6%+	

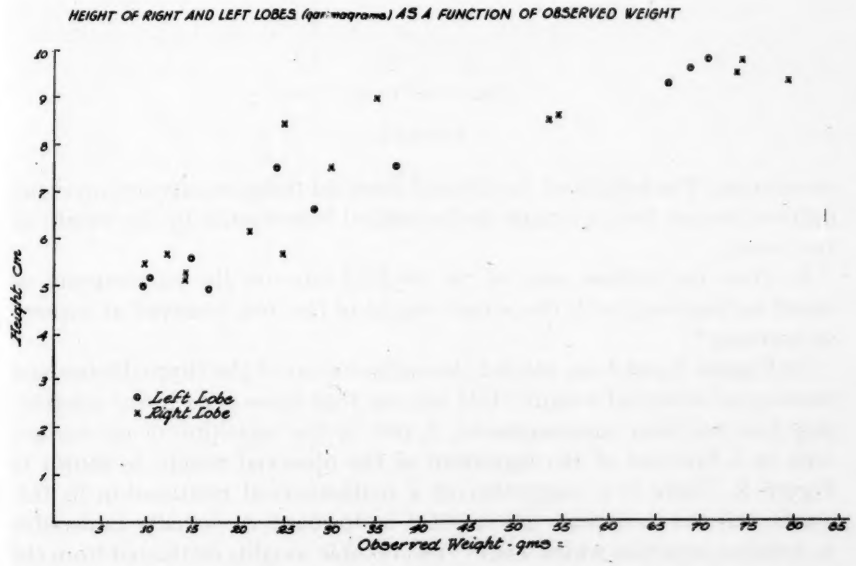
HEIGHT OF RIGHT AND LEFT LOBES (centimeograms) AS A FUNCTION OF OBSERVED WEIGHT
Height cm
Left Lobe
Right Lobe
Observed Weight - gms

FIGURE 1

plotted as a function of the observed weight of the lobe. It is obvious that there is no linear relationship between these data. Marked variation is apparent. In Figure 2 the height of each lobe on the anterior *visual* outline is similarly plotted as a function of the observed weight. The same deductions are applicable to these data. Plots of the logarithm of the height as a function of the observed weight, and of the logarithm of the height as a function of the logarithm of the observed weight, fail to reveal

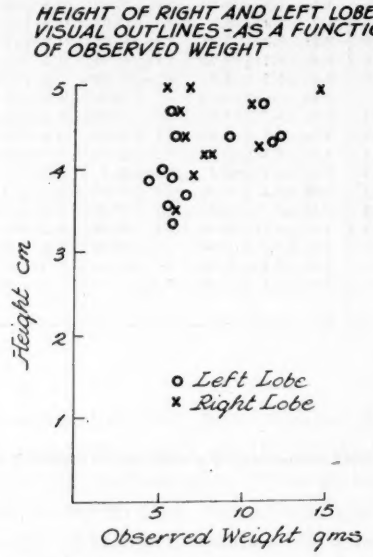

FIGURE 2

correlation. The heights of the thyroid lobes on the gammagrams or visual outlines do not bear a simple mathematical relationship to the weight of the tissue.

b. Does the surface area of the thyroid lobe on the gammagram or *visual* outline vary with the actual weight of the lobe removed at surgery or necropsy?

In Figures 3 and 4 are plotted the surface areas of the thyroid lobes as a function of observed weights. One can see that there is no linear relationship between these measurements. A plot of the logarithm of the surface area as a function of the logarithm of the observed weight is shown in Figure 5. There is a suggestion of a mathematical relationship in this graph, but the variations are so great as to make it virtually impossible to write an equation which would yield reliable weights estimated from the surface areas.

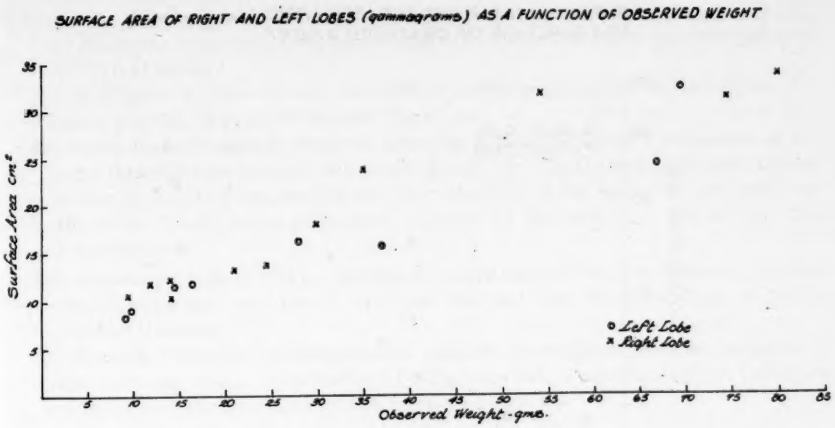
SURFACE AREA OF RIGHT AND LEFT LOBES (gammagrams) AS A FUNCTION OF OBSERVED WEIGHT
Surface Area cm²
Observed Weight - gms.
o Left Lobe
x Right Lobe

FIGURE 3

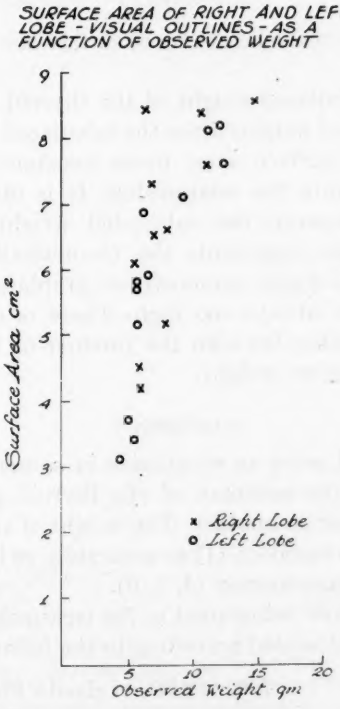
SURFACE AREA OF RIGHT AND LEFT LOBE - VISUAL OUTLINES - AS A FUNCTION OF OBSERVED WEIGHT
Surface Area cm²
Observed Weight gm
x Right Lobe
o Left Lobe

FIGURE 4

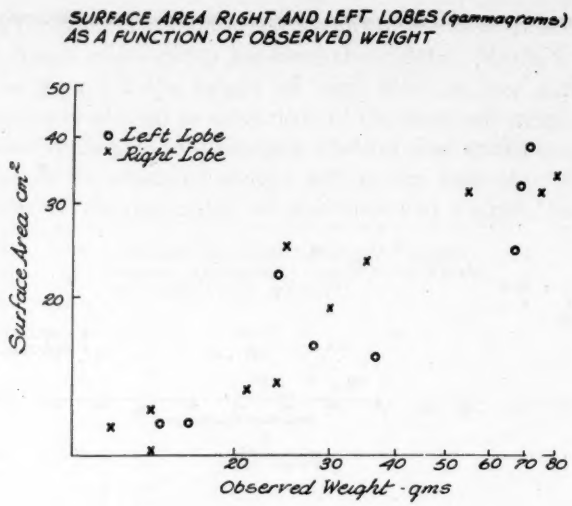

FIGURE 5

c. Does the product of the height times surface area vary with the actual weight?

In Figure 6 the calculated weight of the thyroid gland is plotted as a function of the observed weight. Since the calculated weight is the product of the height times surface area times constant—equation (I)—this would serve to illustrate the relationship. It is obvious that there are marked variations between the calculated weights and the observed weights. The solid line represents the theoretically correct curve. As shown in Table 1, this figure demonstrates graphically the fact that the calculated weights are always too high. There is apparently no simple mathematical relationship between the product of the height times surface area, and the observed weight.

DISCUSSION

The data presented serve to emphasize in a quantitative fashion the marked variation in the anatomy of the thyroid gland, normal or enlarged, from one patient to another. The weight of the thyroid gland cannot be calculated from equation (I) as accurately as it can be estimated on the basis of physical examination (4, 5, 6).

The dose of radioactive iodine used in the treatment of hyperthyroidism in this laboratory is calculated according to the following equation:

$$\text{Dose in microcuries } I^{131} = \frac{7000 \text{ r.} \times \text{Wt. of gland} \times \text{Physical } T\tfrac{1}{2}\ I^{131}}{\text{Max. thyroid uptake} \times \text{Effective biologic } T\tfrac{1}{2} \times 160 \text{ e.r.}} \quad \text{(II)}$$

Where:

7000 Roentgens is assumed to be an average or adequate dose to be delivered per gram of thyroid tissue.

Weight of gland in grams is that calculated from the gammagram or scintogram.

Physical half-life ($T\frac{1}{2}$) of I^{131} is eight days.

Maximum thyroid uptake is obtained, after the administration of 1 microcurie of I^{131}, from the difference between the counting rate over the thyroid area (with the end of the scintillation counter 2.6 cm. from the skin of the neck) and the rate over the lower thigh (same geometry), divided by the counting rate of the dose administered.

Effective biologic half-life ($T\frac{1}{2}$) is that time in days required for the difference between the counting rate over the thyroid area and that over the lower thigh, to fall to one half its value.

160 Equivalent roentgens is the estimated number of roentgens delivered per gram of thyroid tissue when 1 microcurie of I^{131} is evenly distributed throughout this gram of tissue and remains there for full physical decay.

This equation is the same as that derived by Marinelli, Quimby and Hine (7) and employed by many workers in this field.

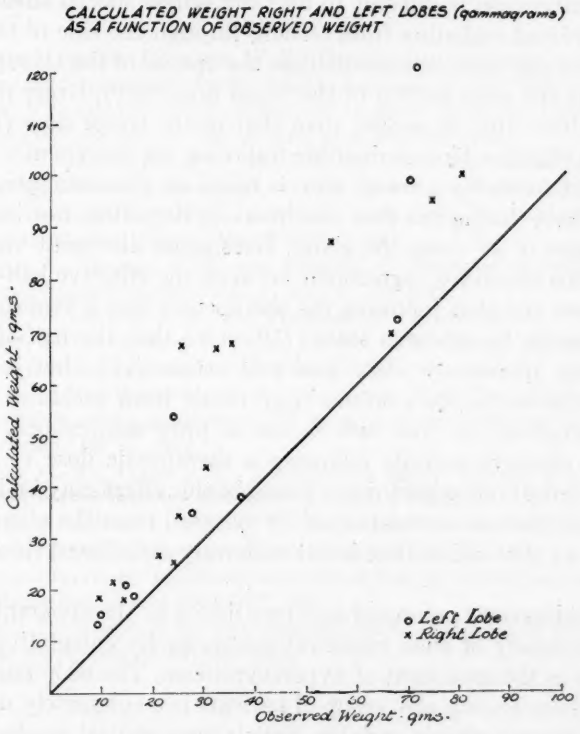

FIGURE 6

Using this equation and assuming all the factors to be correct, except for a 50 per cent positive error (the mean error observed, Table 1) in the calculated weight of the gland, the calculated dose of radioiodine would be twice the expected dose. For example:

$$\text{a. Dose in microcuries} = \frac{7000 \text{ r.} \times 100 \text{ Gm.} \times 8 \text{ days}}{0.70 \times 5 \text{ days} \times 160 \text{ e.r.}} = 10{,}000 \text{ microcuries.}$$

$$\text{b. Dose in microcuries} = \frac{7000 \text{ r.} \times 50 \text{ Gm.} \times 8 \text{ days}}{0.70 \times 5 \text{ days} \times 160 \text{ e.r.}} = 5{,}000 \text{ microcuries}$$

If the patient's gland actually weighed 50 grams, but was calculated to weigh 100 grams, the dose per gram would be twice that intended.

That the uptake of the therapeutic dose is the same as that of the 1-microcurie tracer dose, is based on the assumption that the initial radiation effect—during the first few hours—does not influence the ability of thyroid cells to take up iodine during the subsequent period. The uptake of the therapeutic dose is difficult to measure unless special shielding is designed to prevent radiation from coming through the side of the tube. In the literature one finds statements that the uptake of the therapeutic dose is essentially the same as that of the tracer dose (8, 9), larger than that of the tracer dose (10), or smaller than that of the tracer dose (11).

That the effective biologic half-life following the therapeutic dose is the same as that following a tracer dose is based on the assumption that the radiation effect during the first few hours or days does not influence the rate of release of I^{131} from the gland. Here again divergent views are expressed in the literature. Agreement between the effective half-life following the tracer and that following the therapeutic dose is reported by some investigators (8, 9), whereas others (10) state that the half-life is longer following the therapeutic dose, and still others (11), that it is shorter. These differences in observations may result from technical as well as biologic variations, or from such factors as prior medications. One might expect the effective half-life following a therapeutic dose to be shorter, because of initial radiation injury. Possibly this effect may be balanced or overcome by the reaccumulation of I^{131} released from the gland.

It is known that radioiodine is not uniformly distributed throughout the thyroid.

The considerations presented here cast doubt on the apparent or implied scientific accuracy of such empirical equations for calculating dosage of radioiodine in the treatment of hyperthyroidism. The wide range of tissue radiosensitivity among any group of patients is a completely uncontrolled and as yet uncontrollable variable. Satisfactory clinical results have been reported with radiation dosage varying almost 1,000 per cent (4, 8, 9, 10,

11, 12). Erroneous conclusions will be drawn from the analysis of data concerning the results of therapy, in which average or ideal dosage of radiation per gram of thyroid tissue is calculated. Several investigators agree on this point (4, 10, 12). Although this small series of cases suggests that it would be virtually impossible to write an equation for the accurate calculation of thyroid weight from measurements of the thyroid outline in any one patient, much further study is needed, utilizing the advantages of the newer types and modifications of radiation detection instruments.

SUMMARY

1. Comparison of the weights of thyroid glands as calculated from gammagrams or scintograms, and the observed weights of the same glands removed at surgery or necropsy, revealed a 49 per cent positive error.

2. The assumptions upon which a certain empirical equation are based have been tested and found not to be valid.

3. The problem of dose calculation is discussed.

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Letters to the Editor

MODIFICATIONS OF THE REDDY PROCEDURE FOR 17-HYDROXYCORTICOIDS IN URINE

To the Editor:

In a recent publication, Reddy and associates (1) have described a relatively simple method for urinary 17-hydroxycorticoids which utilizes butanol to extract both the free and conjugated corticoids. Acid hydrolysis of the latter apparently occurs during the Porter-Silber reaction, which is run directly on the butanol extract. The chief limitations of this procedure are the highly chromogenic reactions of many commercial butanols with phenylhydrazine reagent, and the high color found in the butanol extract-sulfuric acid blank. In most urine samples with high values for 17-hydroxycorticoids, these nonspecific reactions, although contributing an undesirably large fraction of total color, have not prevented reproducible results. In urines of low corticoid values and in certain presumably normal urines, however, these interfering colors have constituted such a large portion of the total color as to give, in our hands, either negative or variable results. The purpose of this letter is to report certain modifications of the original procedure which we have found to give consistently good results at all levels of corticoid concentration. The brevity and simplicity of the Reddy procedure make it potentially attractive for many clinical laboratories. We believe the improvements herein reported will enhance its applicability to the diagnosis and management of adrenocortical problems.

The chief modifications are the use of 1) laboratory-purified butanol, 2) a lower acid strength in the Porter-Silber reaction, and 3) a longer incubation period. The sulfuric acid concentration employed is 1.27 volumes of concentrated acid to 1 volume of distilled water, in contrast to the 1.63:1 strength used in the original method.

PROCEDURE

A 40-ml. aliquot of the urine to be analyzed is brought to pH 4.5 by the dropwise addition of glacial acetic acid. Approximately 0.5 Gm. of dry, acid-activated kaolin is added, and after five minutes of gentle mixing the suspension is centrifuged for five minutes at 1,500 rpm, to sediment the kaolin. The urine is decanted and brought to pH 1.0–1.5 by the dropwise addition of 18 N sulfuric acid. A 10-ml. aliquot of urine is then extracted in each of two 125-ml. separatory funnels, using 5 ml. of reagent butanol[1]

for four minutes

TABLE 1. TOTAL 17-HYDROXYCORTICOIDS (MG. PER PERIOD)

Patient, sex, diagnosis	Reddy method	Modified method	Collection period (hrs.)
	Untreated butanol		
G.D. (F) Agitated depression	0	6.7	18
D.C. (F) Sec. amenorrhea	0	13.5	24
M.M. (M) Cushing's syndrome	0	17.5	24
N.G. (F) Cyanosis, congenital heart dis.	1.6	8.1	24
R.D. (F) Cushing's syndrome	6.6	14.4	12
	Treated butanol		
A.P. (F) Normal	0.8	2.0	3
R.M. (M) Normal	1.1	2.0	3
E.V. (M) Whipple's disease, ACTH-treated	16.1	27.4	24
J.B. (M) Hemochromatosis	1.5	1.8	12
K.V. (F) Cushing's syndrome	2.1	7.8	12

for each extraction. The two butanol extracts are pooled in one separatory funnel and any excess water removed. Approximately 1.0 Gm. of anhydrous sodium sulfate is added and the separatory funnel shaken for one minute. After five minutes the sodium sulfate and water are removed and the butanol extract filtered through an 11-cm. #41 Whatman paper into a 50-ml. Erlenmeyer flask containing 0.5 Gm. of anhydrous sodium carbonate.

[1] To date, the most satisfactory preparation has been Mallinckrodt reagent butanol. In all instances, the butanol is re-distilled in the laboratory, collecting only that portion which comes over between 116 and 117° C.

When filtration is complete, the flask is rotated briskly for one minute and immediately 1 ml. of the extract is added to 4 ml. each of the phenylhydrazine-sulfuric acid reagent and the sulfuric acid. After thorough mixing, the tubes are placed in an ice bath until all are ready for incubation. The latter is carried out in a water bath at 60° C. for forty-two minutes. On completion of the color reaction, the tubes are placed in an ice bath for five minutes before transferring the contents to the cuvettes for reading in the Coleman Junior spectrophotometer at 410 mμ. The 17-hydroxycorticoid value of the urine sample is calculated according to the method in the original procedure.

COMMENT

In Table 1 are presented a few selected examples of the contrast in steroid values for urine samples analyzed by the original method and by the method described here. The former procedure often showed no 17-hydroxycorticoids present in urines expected to contain at least normal amounts. As previously mentioned, this result may be attributed to the high optical densities of the sulfuric acid-butanol extract blanks and the phenylhydrazine reagent-butanol reactions. By employing the lower acid strength, purified butanol and a longer incubation period, the nonspecific colors are significantly lowered without materially affecting the development of the specific Porter-Silber color reaction. Since these modifications have been adopted, we have encountered no inexplicable zero values and have noted more uniform results, which have been well correlated with the various clinical diagnoses.

The use of kaolin has been helpful in overcoming the emulsions which form during the extraction with butanol. Kaolin adsorbs the urinary proteins known to have emulsifying properties, and careful studies have shown no loss of 17-hydroxycorticoids following its use. It makes centrifugation unnecessary for the separation of the butanol from the extracted urine, and it also permits the removal of gonadotropins from the urine sample to be analyzed for 17-hydroxysteroids and 17-ketosteroids.

Standard curves, absorption spectra curves and recovery experiments with this method are similar to those reported in the original publication.

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USE OF CHLOROTRIANISENE (TACE ®) FOR SUPPRESSION OF POSTPARTUM LACTATION

To the Editor:

No method yet reported for the suppression of lactation in the postpartum patient has proved to be entirely effective. The use of androgens, as well as various estrogenic substances, has resulted in a varying percentage of suppression, but each method has left something to be desired.

It has been been the observation of this writer for a number of years that diethylstilbestrol, used in diminishing dosage, was a fairly efficient method of suppression of painful breast engorgement while the patient was in the hospital, but that patients so treated would frequently telephone after they had been at home for several days, stating that the breasts had become painfully engorged. In some cases, the engorgement became so painful that codeine was required for symptomatic relief. It appeared that as often as not, the role of the diethylstilbestrol was simply to delay the appearance of painful engorgement until after the patient's return home, at which time she was least capable of coping with an additional discomfort of the puerperium. It was assumed that a relatively rapid drop in estrogen level after the discontinuance of therapy was responsible for this delayed engorgement.

The introduction of TACE® (chlorotrianisene) seemed to offer a means of allowing the estrogen level to drop at a more gradual rate. This drug is a synthetic estrogen which has physiologic effects similar to those of other synthetic and natural estrogen preparations; but it also has the additional quality of being stored in body fat (1). The possibility of avoiding the late "flare-up" of breast engorgement in the postpartum patient led to a clinical trial of TACE in puerperal patients.

Sixty consecutive patients were included in this series. The administration of TACE was begun within the first postpartum day. One 12-mg. capsule was given four times daily for seven days. Snug breast binders or brassieres were worn in most cases. The results of therapy were rather critically evaluated, the patients being followed for at least eight weeks.

RESULTS

As may be noted from Table 1, among the 60 patients, 45 (75 per cent) remained entirely free from any symptoms of engorgement, whereas 11 (18.3 per cent) noted only mild engorgement which did not require any treatment. Only 4 patients (6.7 per cent) had enough discomfort to require aspirin or ice bags.

Lactation was completely suppressed in 50 cases (83.3 per cent), but

TABLE 1. SHOWING THE INCIDENCE OF PAINFUL BREAST ENGORGEMENT AND LACTATION IN 60 PATIENTS WHO RECEIVED TACE

	No. of patients	Percentage
Painful engorgement		
None	45	75
Mild; no treatment required	11	18.3
Severe; required treatment	4	6.7
After discharge from hospital	None	
Lactation		
None	50	83.3
1–3 days	4	6.7
4–7 days	3	5
8–14 days	1	1.7
Over 14 days	2	3.3

in 10 cases there was some degree of lactation present for from one to twenty-one days. In only 2 cases did the duration of lactation exceed fourteen days: in these instances the administration of TACE appeared to be almost without effect, inasmuch as both these patients experienced several days of mild breast engorgement followed by lactation, which was prolonged in spite of the fact that the infants were not put to breast. Most instances of lactation were noted after TACE was discontinued. No additional TACE was given to any patient after the reappearance of symptoms, inasmuch as it was desired to evaluate the results of a single course of the drug.

TACE appears to compare most favorably with other drugs used to suppress lactation. Eight of the patients in this series stated that TACE was more effective than the drug employed with a previous pregnancy for the same purpose. Interestingly enough, 5 of these 8 patients were "partial failures" with TACE, having experienced either engorgement or lactation, or both. It would appear that some patients may not be susceptible to hormonal suppression of lactation, regardless of the drug used.

In common with other similar preparations, TACE was not effective when started too late in the postpartum period. In 3 patients (not included in this series), who were started on TACE either at the third or fourth postpartum day, the drug proved ineffective in altering the symptoms.

Although this writer was unable to achieve the excellent percentage of successes which was reported while this study was in progress (2), he does feel that TACE has definite superiorities over the short-acting estrogens.

Perhaps the most gratifying observation in this series was the fact that not one patient telephoned during the first week after discharge from the hospital to complain of painful breast engorgement. All symptomatic breast engorgement, of whatever degree, occurred during the patients' postpartum hospital stay, and there was no mastitis.

It is difficult to assay bleeding quantitatively during the postpartum state; however, in the current series there was no evidence that patients who had received TACE therapy had a bleeding history which differed in any degree from that of other postpartum patients.

SUMMARY

TACE, a synthetic estrogen which is stored in body fat, appears to be a satisfactory drug for the suppression of lactation post partum. Of 60 patients studied, 93.3 per cent required no active treatment for painful engorgement, and 83.3 per cent were free from any evidence of lactation.

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DETECTION OF CORTICOID CONJUGATES IN HUMAN BLOOD*

TO THE EDITOR:

The method of estimating 17-hydroxycorticosteroids in blood, described by Nelson and Samuels (1), has been most helpful in elucidating a number of problems in relation to adrenocortical function. As described, only the free (unconjugated) compounds are measured by this technique. Evidence has been accumulated in this laboratory indicating the presence of conjugated corticoids in human blood.

The essential procedure employed was that of Nelson and Samuels, with exceptions as noted. Serum was first extracted directly with chloroform in the usual manner. A duplicate specimen was incubated with 1,000 units of glucuronidase (Warner Institute, New York) per ml. of serum and sufficient acetate buffer to establish a pH of 4.5, at 37° C. for twenty-four

* This work was made possible by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

hours. Earlier trials indicated that smaller quantities of glucuronidase were not as effective. When direct extracts of serum so treated were chromatographed, the final color reaction was influenced by many nonspecific contaminants, so that it was not possible to determine the quantity of corticoids present by the reaction with phenylhydrazine. Therefore, after incubation of the serum, it was dialyzed against 20 volumes of water for from forty-eight to seventy-two hours. The water was then extracted with chloroform, and the residue thereby obtained was chromatographed in the usual way.

TABLE 1

Subject	Blood 17-hydroxycorticoids (gamma per 100 ml. serum)	
	Free	Glucuronidase hydrolysis
1. Normal female, 57 years.	6.1	18.0
2. Normal male (fresh pooled blood)	6.1	13.2
3. Female (obesity), 55 yrs.	0.7	4.2
4. Normal male, 30 yrs.	0	9.6
5. Pooled blood (after standing 24 hrs.)	0	21.9
6. Normal male, 32 yrs. (90 mins. after 200 mg. of cortisone orally)*	44.3	181.0
7. Adrenal hyperplasia		
(36 hrs. after 50 mg. of cortisone i.m.)	10.6	21.2
(45 mins. after 200 mg. of cortisone orally)	50.8	56.0
8. Female, 6 yrs. (adrenal tumor, Cushing's syndrome)†	34.2	44.7

* Repetition of this experiment with a different system of extraction, which is being studied by Doctor George Clayton, yielded similar results.

† The patient died three days after this determination. Autopsy revealed extensive infiltration of the liver with neoplastic metastases.

Preliminary results are presented in Table 1. The levels of corticoids in serum extracted directly and in duplicate specimens treated with glucuronidase are shown. Previous conclusions based on the level of free corticoids should be re-evaluated in the light of the apparent presence of conjugates in blood, which may be in especially high concentration after the administration of cortisone.

The material measured after glucuronidase hydrolysis and dialysis, on treatment with phenylhydrazine, yielded measurements at 370, 410 and 450 mμ which conformed more with those for pure compound E than with those obtained by the usual procedure. Dialysis alone of unhydrolyzed

serum did not increase the yield of corticoids. Some preliminary studies with acid hydrolysis followed by dialysis revealed less satisfactory yields. Further studies with the method for hydrolyzing conjugated corticoids in blood are being conducted.

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Teaching Clinic

PSYCHOLOGIC CHANGES IN 3 CASES OF ADDISON'S DISEASE DURING TREATMENT WITH CORTISONE

INDIVIDUALS suffering from Addison's disease frequently exhibit personality changes of mild to moderate degree and more rarely a psychosis, usually with paranoid trends (1–5). A recent editorial in this journal comments on these changes (6). The most common psychologic features in untreated cases are apathy, negativism, querulousness, irritability and depression. The introduction of desoxycorticosterone provided maintenance therapy which was a striking improvement, permitting many patients to carry on at a level of activity that was gratifying, if still less than normal. Some of them, however, continued to show psychologic deterioration despite a reasonably satisfactory physical status. The addition of cortisone to the therapy of the Addisonian patient resulted in a much improved restoration of the capacity for activity and adaptation to a variety of stresses, though transient mental excitement was noticed in the early days or weeks of treatment (7). Similar mood changes, and indeed psychotic reactions, have been described for patients with other diseases during cortisone therapy (8, 9, 10). Little mention has been made in the literature of psychotic reactions accompanying the use of cortisone in Addison's disease. Thorn *et al.* (7) mention 1 case, and 3 others seen by us have been briefly described (6, 11). The fact that we saw these 3 within a relatively short period makes us suspect that the incidence of such reactions is probably higher than reported, and that the Addisonian patient is peculiarly susceptible to an amount of the drug well tolerated by patients with other diseases. The initial dose in our 3 cases was two or more times the 25-mg. daily dose used by Thorn *et al.* (7). However the onset of psychoses in 2 and the exacerbation of a pre-existing psychosis in the third, occurred within seventy-two hours of initiating cortisone therapy, when less than a total of 300 mg. had been given. The correlation of information on these 3 cases and more extensive detailing of the observations on 2 of them may excuse the repetition of some of the data already in print.

Case 1

This 38-year-old man, a patient of Drs. A. E. Moll and M. Bourne at the Montreal General Hospital, had both diabetes and Addison's disease. It was felt that he might benefit still further from cortisone and it was accordingly administered. After receiving 250 mg. in a three-day period, he became stuporous and resistant. Blood levels of sodium, potassium and sugar were within normal limits. Cortisone was stopped but for two days more he was confused, unresponsive and speechless and had to be tube fed. On the third day aqueous cortical extract, desoxycorticosterone acetate (DCA) and testosterone were given. Not till the fourth day did he speak, and then only briefly. On the fifth day after the onset of this catatonic-like state he was sufficiently in contact to confess that he had had a desire to commit suicide. Improvement continued and later study showed a pre-existing neurotic adjustment.

The development of stupor is rather rare in cortisone- or ACTH-treated patients. Glaser (10) reports such a case and we have seen another. Both occurred in women suffering from dermatomyositis and both had received very large amounts of hormone over several weeks when the reactions occurred.

In the next patient to be described, cortisone therapy was accompanied by an exacerbation of a psychosis which developed during the course of the maintenance treatment with DCA. Almost all the information was obtained by nonpsychiatrically trained residents and staff.

Case 2

This woman, who was 29 years of age when first seen at the Royal Victoria Hospital on the service of Dr. J. S. L. Browne in September 1944, gave a history of an induced delivery for pre-eclampsia at the age of 22. Following this she received a salt-free diet for a month and nursed her baby for three months—two good lines of evidence against adrenal disease at that time. She was described as a capable person with a high school education, who tended to be apprehensive and worried about things which she feared might happen. No other pregnancies occurred, though desired. She was well until March 1944, when her legs felt stiff after bowling and she noticed dyspnea on walking. Pigmentation also became noticeable at that time and she had anorexia, weakness, dizziness and amenorrhea which lasted for four months. She lost the desire to work. In the ensuing months nausea and vomiting developed. A diagnosis of Addison's disease was made in a local hospital in June 1944, and she was given DCA and salt. Following this therapy her breasts became larger, but clinical improvement was insufficient, so she was sent to Montreal where the diagnosis was confirmed on clinical and laboratory grounds. It was noted that the pubic hair was thin. The treatment instituted was NaCl (3 Gm.), DCA, methyltestosterone, and a high protein diet. She carried on at a reduced level of activity, complaining at times of weakness, fatigue and lethargy, but she was oriented and cooperative. The speech was observed to be slow. She returned two or three times a year for check-up and adjustment of therapy. The implantation of pellets of DCA in September 1948 was followed by improvement for eleven months; then weakness became more marked and she returned for more pellets. Just prior to this admission in September 1949, she began for the first time to speak of voices talking about her and telling her to do certain things. Because of the fullness of her breasts and a missed period

she thought she might be pregnant, but she was not. Two 125-mg. pellets of DCA were implanted September 24. Five days later she received penicillin for a cold. Next day she was emotionally upset, crying and saying that her daughter was on the ward and that the patients were persecuting her and her family. Lipo-adrenal extract (Upjohn) was given daily for a week, during which time she lost her paranoid ideas, felt better and showed a livelier facial expression. Five days after stopping this preparation containing C-11 corticoids paranoid ideas were again expressed. In February 1950, five months after the last pellet implantation (much before the expected time of need for replacement), the patient returned complaining of weakness, tiredness and amenorrhea. There was a return of hallucinations. The blood potassium level was possibly slightly elevated at 5.5 mEq./L. Psychologic tests were performed on March 1 and the report read "This then is a rigid, impoverished, meticulous and perfectionist individual who presents evidence of considerable anxiety, sexual conflict and possible paranoid trends." On March 8 a note stated that she had had no paranoid ideas for a few days. On March 13 she was described as anxious and depressed over paranoid delusions. Shortly afterwards she said they were imaginary, and wept. Cortisone was started that day in a dose of 50 mg. daily. In the next twenty-four hours she felt better and was cheerful. On March 15 she said that she thought she had had a minor operation during the night. On March 16, after a total of 150 mg. of cortisone in three days, she was extremely deluded and agitated, and would not talk freely. However, she admitted she thought she heard her husband breathing beside her during the night, though he would not answer her. The next day, after 200 mg. of cortisone, she was still agitated, saying she heard the head nurse make a statement about machines. In the succeeding three days she cried periodically, was tense, depressed and paranoid, hearing people talk about her and having hallucinations about her husband's voice. Once she woke in the night to ask "Is my whole side open?" Despite these adverse developments the dose of cortisone was raised from 50 to 100 mg. daily and in four days she felt more at ease, saying that the tests and new drug caused her to imagine things. Next day she felt better than at any time since the onset of her illness. The ideas were gone and she said they were imaginary. She was discharged on 50 mg. of cortisone twice weekly and 6 Gm. of NaCl daily.

The mental deterioration in this woman while on DCA therapy is of considerable interest. If is unfortunate that a psychologic test was not obtained initially. At the time of testing, though she seemed relatively free of mental symptoms, she was not examined psychiatrically. An incidental observation of interest is the alleged increase in breast size following DCA therapy. One of us has observed gynecomastia in an Addisonian man to whom DCA was administered for a long period. The improvement of the mental state with the increase in the dose of cortisone seems anomalous.

The case to be described next was more fully documented than the previous two. It serves to illustrate that the medical clinic may sometimes be a more fruitful place for observing psychologic changes incidental to therapy than the psychiatric hospital, where obvious physical disease and the associated therapy may be less prominent.

Case 3

A white female, the fourth of nine children, was born in Eastern Canada and was healthy as a child, having only chickenpox and measles. When she was 10 years old her mother died in childbirth and some years later her father remarried. She stopped school in grade 10 at the age of 16, left home because of stepmother trouble, and went into

domestic service. As a girl and young woman she was always able to fight for herself, though she was always underweight and visited many doctors on this account and for what was described as anemia.

Menarche occurred at 16. A romantic affair led to pregnancy and a Cesarian birth at the age of 20. The baby, a girl, was adopted by strangers rather against her wishes. When 27, she met a man ten years her senior, a rather passive but considerate person employed as a clerk, whom she married. Shortly after this a dilation and curettage was performed for uterine bleeding and hemorrhage. About this time hot flushes and headaches developed, and soon the menses ceased. Otherwise she felt well and lived an active life, worked in an office, kept house and walked a lot. Libido, which had never been very strong, disappeared at about the age of 30. Four years later she had one more menstrual period, her last.

The presenting illness dated from the age of 32, in December 1940, when she was ill briefly with a dry pleurisy, and as part of her convalescence, went skiing. This resulted in fractures of the right tibia, right fibula and left tibia, without displacement.

January 9, 1941 (first admission): The fractures were set, and no other physical abnormalities were noted except her weight—87 pounds, with a height of 5 feet 3 inches. The B.P. at this time was 120/80. She was discharged after twenty-four hours but was in a cast for four months.

The patient attained her greatest known weight of 97 pounds in the summer months of 1941, but noticed an abnormal tan on her hands and a brown spot on the face, which disappeared during the subsequent winter. The next adverse development occurred in June 1944, when she had an attack of diarrhea and vomiting which kept her at home for ten days. This recurred in August and September and again in January, 1945. Since she gave a history of seeing small $\frac{1}{4}$-inch white worms in the stools for three days before each of these bouts, intestinal parasites were suspected.

February 14, 1945 (second admission): The patient came in for investigation. Inquiry elicited little more information except that there had been a slight weight loss and monthly attacks of dizziness for the preceding five months, which she complained her physician had been unable to explain. Physical examination showed a thin woman weighing 80 pounds, described as not well developed but adequately nourished, and with poorly developed breasts. The genitalia were described as healthy, but atrophic (postmenopausal). There was no lymphadenopathy; the B.P. was 120/80, and the pulse 84. The blood showed significant deviation, with a hemoglobin of 72 per cent; R.B.C. 3.6 million; W.B.C. 7,000; lymphocytes 29 per cent; and eosinophils 15 per cent. No free HCl was found in the stomach; the B.M.R. was −19 per cent. X-ray examination of the chest showed thickened apical pleurae; there was nothing abnormal in the colon. The diagnosis was secondary anemia with hypothyroidism. It was pointed out that the patient had always been slight and had visited many doctors for her anemia and underweight.

February 14, 1947 (third admission): The patient suffered a compression fracture of the first lumbar vertebra when thrown on her back while boarding a streetcar. The B.P. was 134/80, and she was described as unchanged from her previous admission, except for the fracture. She was in a cast for one hundred days after this, and during that time she showed a slight personality change, in that she lost patience for doing needle work which she had previously enjoyed. She also became irritable and tired. The B.P. in May 1947, when the cast was removed, was 106/84. There was brownish patchy pigmentation on the backs of her hands and the base of her fingers. The blood picture showed: Hb 82 per cent; R.B.C. 4.1 million; W.B.C. 7,000; lymphocytes 35 per cent; and eosinophils 3 per cent; the sedimentation rate was increased to 27.

October 27, 1950 (fourth admission): The patient, now 42 years old, was admitted

complaining of dizziness, weakness, nausea and vomiting, and shortness of breath for the previous three weeks. She said pigmentation of the hands, face and legs had been present for ten years; more dark spots had appeared on her face in December 1949. For the preceding two months she had received adrenal cortical extract injections from her outside doctor. Her weight was 76 pounds, B.P. 88/68, and blood examination showed: Hb. 80 per cent, R.B.C. 4.1 million; W.B.C. 12,000; lymphocytes 31 per cent; eosinophils 7 per cent; and sedimentation rate 40. Urinary corticoids were 29 glucogenic units per twenty-four hours (normal) and 17-ketosteroids 0.85 mg. per twenty-four hours. The concentration of blood sodium was 120 mEq., and of potassium, 4.8 mEq./L. The B.M.R. was −14 per cent. ACTH did not increase the 17-ketosteroids significantly, but raised the chemical corticoids from 0.2 mg. to 0.4 mg. per twenty-four hours. Diagnosis: Addison's disease. She was discharged on NaCl and testosterone.

After ten days at home the patient became edematous, so the salt was reduced to 1 tablet (1 Gm.) per day. Symptoms of nausea, vomiting and weakness soon recurred, however, and salt was increased to 2 Gm. a day, with no change in the clinical picture. Testosterone was stopped about the middle of January.

February 6, 1951 (fifth admission): The patient was weak, anorexic, lethargic, drowsy and nauseated. She was cooperative but complaining, said her memory was poor and that she had been more nervous and irritable in the previous week. She was thin and poorly developed. Pigmented areas were present on the extremities, head and neck. Hair was absent in the axillae and scanty on the extremities. The breasts were atrophic. She weighed 82 pounds and her B.P. was 80/55. The blood showed: Hb. 86 per cent; W.B.C. 10,800; lymphocytes 42 per cent; eosinophils 10 per cent; and sedimentation rate, 39. The blood level of sodium was 120 mEq. and of potassium, 5.1 mEq./L. Results of the water test were positive; the glucose curve was flat. She was given 5 Gm. of NaCl daily. In the following nine days the patient became weaker and vomited small amounts occasionally. She ate little, became more apathetic and depressed, was extremely lethargic and hiccoughed frequently. She complained of coldness and numbness in her hands and feet.

February 15—The blood sodium level was 118 mEq. and potassium 7.7 mEq./L. The blood sugar level was 97 mg. per 100 ml., after a long fast. She excreted a very small quantity of urine. The B.P. was 66/56 and the pulse was barely obtainable. Glucose-saline was given intravenously (1,500 ml.) and adrenocortical extract (ACE)(65 ml.)

February 16—The patient was considerably improved. The pulse was of good volume and the B.P. was 76/36. She was quite alert and ate a fairly substantial breakfast.

February 17—She vomited small amounts of food. The systolic B.P. was 80. She complained of dizziness and impairment of hearing.

February 18—B.P. 72/56. The patient was weaker. She received glucose-saline (1,000 ml.) intravenously and ACE (75 ml.), with improvement in a few hours in B.P. and clinical condition.

February 19—Cortisone (125 mg.) was given at 10:25 a.m., followed by 25 mg. at 2:00 p.m., 8:00 p.m., and 12:00 p.m. She felt stronger by evening.

February 20—Cortisone (50 mg.) was given in 4 doses. She slept well and was cheerful.

February 21—Cortisone (50 mg.). The blood sodium level was 130 mEq. and potassium 4.5 mEq./L. On this, or the following night hallucinations first appeared. She said she saw brilliant colors, orange, red and blue shapes, a fan made of ostrich feathers, heads of animals and a delicate orange parasol. She also noticed a return of sexual feelings, the first she had had in more than ten years, with the exception of one occasion two months previously, when at a party a man made some suggestive advances.

4 February 22—Cortisone (50 mg.). The systolic B.P. was 80. Appetite was improved but only fair. The patient had felt extremely well since beginning of cortisone therapy, and was actually euphoric at times. The extremities were warm for the first time in years.

5 February 23—Cortisone (37.5 mg.) was given in 4 doses. She complained of being hot, with tingling sensations in feet. She felt shaky and apprehensive, though she slept well.

6 February 24—Cortisone (37.5 mg.). Improvement continued, with a gain of 2½ pounds. One half hour following ingestion of cortisone on an empty stomach, there was a sense of warmth and tingling in the extremities which radiated to the face and head at times; it was accompanied by perspiration and lasted several hours, leaving the extremities cold. This was confirmed objectively. These symptoms were particularly marked after the midnight dose and were accompanied by a great increase in mental activity. A sleeping pill was necessary to control the "push" caused by the midnight dose of cortisone.

7 February 26—Cortisone (27.5 mg.). She reported hearing celestial-like music after the midnight dose of cortisone.

8 February 27—Cortisone (21 mg.). The euphoric state had become more marked during the previous few days, until on this date she displayed hypomanic behavior during rounds, describing, with histrionics, the sensory changes which followed the dose of cortisone, and stating that she was a "human dynamo" since starting therapy. She later revealed to the interne the story of her rigid dominating stepmother and the daughter born to her at the age of 20 out of wedlock. She was emotional at this point, and described her marriage as not very satisfactory sexually. She spoke of her awakened sex desire since cortisone therapy, and indicated sexual fantasies directed towards the interne.

9 February 28—Cortisone (12.5 mg.). She was still hypomanic and having hallucinations, though she seemed somewhat more relaxed with the reduced dose. She said that the parasol spoken of on February 21 had to do with her daughter, who appeared before her several nights in bridal dress to ask her approval. This made patient happy.

Psychologic examination (*Rorschach test*)—In summary, the test showed an intensely disturbed, fearful woman showing lack of critical judgment and an overriding anxiety and preoccupation with pregnancy and sexuality and little fantasy reserve. She referred to card 10 as "an atomic bomb that's created complete havoc—bodies have been blown to pieces, scattered, burned, dead." Phraseology was in keeping with her comparison of the effects of cortisone treatment with the force of an atomic explosion. Reality contact was good, as shown by her ability to see popular responses on cards 8 and 10, but her consuming anxiety and turmoil took precedence.

11 March 2—Cortisone (12.5 mg.). She described again her feeling with each dose. She was in the process of reading three books, explaining that many people needed her help and were asking for it. They were predominantly men.

12 March 3—Cortisone (12.5 mg.).

13 March 4—Cortisone (12.5 mg.).

14 March 5—Cortisone (12.5 mg.). She stated the dose had been increased. This was not so. She had colored dreams and hallucinations, and was much less active.

15 March 6—Cortisone (12.5 mg.). The blood pressure was gradually declining. She showed somatic preoccupation, saying that she felt weak, bloated and tired and that there was sensation of pressure in breasts. She felt everything more acutely, *e.g.*, pain from needles. Her taste was keener, as was also her desire for perfection, which was always a characteristic. She said that she has been exploited enough, and that we should get another Addisonian to experiment on. She saw moving forms in the room at times, beseeching her, and felt that she had the power to influence others. She was antagonistic

to cortisone and reluctant to take it, comparing it to atomic power, which is green like the color produced by cortisone. She believed cortisone could destroy her as quickly as atomic power.

March 8—Cortisone (18.7 mg.). The dose was increased, as the patient was nauseated and dizzy, with a blood pressure of 88/74.

March 9—Cortisone (25 mg.). She claimed that she felt on a more even keel and at peace.

March 10—Cortisone (25 mg.). She stated that she would menstruate again and have a baby, but that she felt a weakening in her ovaries. The B.P. was 110/70.

March 11—Cortisone (25 mg.). She was tense, depressed, exasperated, and had visual hallucinations.

March 12—Cortisone (12.5 mg.). The B.P. was 112/80. She felt trapped, and was questionably suicidal because her sex ideas could not be controlled.

March 14—Cortisone (12.5 mg.), DCA (2.5 mg.). She wanted to sleep a lot but did so for only short periods.

March 15—DCA (2.5 mg.). She had slept well, and conversed quietly on any topic during the day. She read a good deal, was less tense, and more cheerful and relaxed.

March 16—Cortisone (6.2 mg.), DCA (2.5 mg.). Her mood fluctuated from depression to occasional elation, and she was found sleeping frequently during the day. Sex feelings were less troublesome since DCA was started.

March 17—Cortisone (3 mg.), DCA (2.5 mg.). She was depressed, and refused DCA on two occasions; after a short interview she acquiesced. Emotional lability continued. The B.P. was 90/50.

March 18—Cortisone (6.2 mg.), DCA (4 mg.). A crisis seemed imminent, hence the dose was raised. The B.P. was 98/70. She felt better following a visit from her brother.

March 19—Cortisone (12.5 mg.), DCA (4 mg.). Mood swings continued, and depression predominated. She talked of suicide, and described a pit full of hate and fear into which she was falling. She felt that there was a competition between cortisone and DCA—that cortisone was tending to build up her mental processes while DCA was tending to bring them down. She said she was weak but told another examiner that she felt wonderful and had visions of men in the room. The nurse said she was negativistic.

March 20—Cortisone (15 mg.), DCA (4 mg.). She continued depressed and negativistic. She refused NaCl and breakfast and required a 45-minute interview to persuade her to take them. Chemical analyses of the blood showed the electrolytes to be within normal limits.

March 21—Cortisone (25 mg.) in divided doses. No DCA on this date or thereafter. There was a marked improvement in mood, and less desire to sleep than when taking DCA. She was vivacious at all times and wanted to cooperate. She said she used the bed as a refuge while taking DCA, and did not like the loss of libido under this form of treatment. Libido had returned and she felt that she could find satisfaction somewhere.

March 22—Cortisone (25 mg.).

March 23—The improved affective level was maintained. The B.P. was 110/70. She was looking forward to going home, and was discharged on cortisone (25 mg. in divided doses) and NaCl (2 Gm. daily).

The patient did well after her return home, gained weight and had no serious relapse. She was more vigorous, gay, alert and interested when seen in the spring of 1953, than at the time of her discharge two years previously. The Rorschach test was repeated.

Psychologic examination—The psychologic tests showed little change in the Rorschach picture. The content remained much the same, though perhaps a little more toned down,

less flamboyantly expressed, less symbolistic. Her preoccupations with birth and pregnancy persisted. She seemed to be a little less ready to react to affective stimuli (color) and a little more restrained and controlled. The figure drawings were essentially the same (*i.e.*, featureless, naked, sexually undifferentiated figures) but expressed with somewhat greater sense of freedom, forcefulness, assurance and activity. "This woman presents a most interesting problem from the point of view of diagnosis. As one talks with her, she seems alert and in excellent contact, though perhaps a trifle facilitated. On the other hand, if one were to look at her test results without knowledge of the case, one would be inclined to label her a schizophrenic—for much bizarreness, accidental thinking and poor judgment is exhibited. It is regrettable that this woman was not brought to our attention before cortisone was started, for we have no baseline against which to measure her."

DISCUSSION

If the optimum advantage is to be gained from psychologic tests in judging effects of treatment on mental status they should be used before treatment is started. The mental changes which developed in these cases during cortisone therapy were not anticipated. It is to be hoped that others may profit by our experience. The time of appearance of psychologic changes was similar in the 3 cases reported, being roughly three days after starting cortisone. This is much earlier than usually observed for the development of psychosis in patients with other types of disease (9, 10). The doses received in the three-day period were 250, 150 and 300 mg., respectively—not large for other diseases but considerably more than employed by Thorn *et al.* (7) in treating Addison's disease. It is not improbable that the Addisonian (who suffers from a severe defect in steroid secretion) experiences a more marked stimulus with hormonal treatment than do other individuals, when his tissues are exposed to a sudden change in the hormone level from almost nil to a greatly increased concentration. This factor, or tissue changes associated with adrenal insufficiency, may account for the apparent susceptibility to mental changes. Case 3 gave suggestive evidence that DCA had a sedative-like action, dampening the cortisone-induced aggression, sexuality and lability. The patient said there was a competition between them. Without trying to prove the perspicuity of this observation it is worth quoting that Woodbury *et al.* (11) suggest that DCA and cortisone "compete for the strategic loci in the cell," as an explanation for their finding that DCA renders rats less sensitive and cortisone renders them more sensitive to seizures induced by electroshock (13). In Case 3 it also appeared that the "push" resulting from the midnight dose of cortisone was controllable by a sedative. One feature of this woman's emotional disturbance was the extreme lability during a 24-hour period. Different observers, seeing her at different times, did not find the same intensity of mood or mental content. This variability was apparently more a manifestation of the toxic-like psychotic state than of the accuracy of different observers or the patient's reaction to them.

The observations contained in this report indicate the opportunity available to medical men for making studies on the psychologic effects of treatment. The results may be unexpected but they are nonetheless of critical importance, especially in appraising new forms of therapy. It is also suggested that cases of Addison's disease constitute appropriate test subjects for the study of the relationship between steroid hormones and behavior.

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Editorial

LABORATORY PROCEDURES FOR CLINICAL ENDOCRINOLOGISTS

FREQUENTLY advances in our understanding of disease processes, and improvements in our ability to manage therapy and occasionally in our ability to diagnose disease, depend on the evolution of specific chemical methods for determination of the concentration of metabolites, of administered drugs or of agents (such as enzymes or hormones) which influence the concentration of metabolites. Many methods for the determination of drugs, metabolites and their products, *e.g.*, those for sulfonamides, glucose, amino acids and carbonic acid, though still capable of improvement, are relatively satisfactory. Adequate procedures are available also for the measurement of many enzymes such as the phosphatases. However, methods for the chemical determination of hormones are still relatively unsatisfactory. That this is not due to lack of effort will be apparent from examination of the proceedings of a recent conference in Shrewsbury as recorded in Volume IX of Recent Progress in Hormone Research. This volume deals almost entirely with methods for the determination of one group of hormones, the steroids.

One reason for the unsatisfactory status of these chemical methods is the fact that the hormones are in relatively low concentration in the preparations to be tested; yet low concentration has not been an insurmountable obstacle in the development of methods for the measurement of vitamins. Another reason is the fact that the molecules of most hormones are complex and large; yet the complexity of the large protein molecules of enzymes has not stood in the way of measurement of relative concentrations of enzymes. Due to the excellent work of Sanger (1) the sequence of amino acids in the insulin molecule is now known, but there is still much to learn about the detailed structure of this and other protein and polypeptide hormones. Though the structures of many steroids are believed to be well established, their chemical and physical properties and those of their reaction products are still being investigated (2). Once these details are better known and more widely appreciated, many of the chemical and physical reactions helpful in the development of improved assay methods will be more readily discernible.

Perhaps the chief reason for the present inadequacy of methods for assay

of hormones is our lack of an understanding of the mechanism of action of hormones. Just as a knowledge of the role played by vitamins in the control of the enzyme reactions of intermediary metabolism helped in the logical development of assay methods for vitamins, so too an understanding of the mechanism of action of any given hormone may make apparent a sensitive and suitable *in vitro* model for rapid assay of that hormone. Perhaps this will be by determination of a rate, just as the relative amount of an enzyme can be measured by the rate of the reaction which it catalyzes.

This may be an appropriate place to speak a word of encouragement to methodologists. Those who develop simple, reproducible, specific and accurate methods for the measurement of biologic products and processes are the indispensable but often unsung heroes of our science. Those who can resist the urge to apply a second-rate method in their eagerness to reach what others often fool themselves into thinking is an answer, and especially those who take time to evolve satisfactory methods of measurement rather than to pursue the more glamorous work which involves the application of the methods, are probably making the more significant contributions. Although the usefulness of a method becomes apparent through its application, and although individuals are needed who will properly apply the best available methods, whose who are qualified to apply methods are relatively more numerous than those discerning individuals who can, from a consideration of the chemical and physical properties of a substance, evolve a specific, sensitive and accurate method for its determination. The need for fundamental investigation of chemical properties preliminary to development of improved methods is perhaps presently greater within the field of endocrinology than in any other division of clinical chemistry. It is important that talent be directed to this prerequisite work and to the development of improved methods for the measurement of hormones.

However, for perfect methods, clinicians and other investigators would have to wait indefinitely. Obviously, they must select the available method best suited for their work. The criteria of analytic methods for clinical chemistry have been outlined in considerable detail elsewhere (3), but what follows may serve in summary as guiding principles in the selection and use of the most suitable methods presently available, as well as in the development of improved methods.

The type of method to be selected should depend upon the use which is to be made of the data to be collected. For fundamental research work, maximum specificity and sensitivity are especially desirable attributes. Elaborate preliminary fractionations and subsequent use of complicated and expensive equipment may be required to answer certain questions which involve metabolism of, or metabolism controlled by, hormones.

Such methods may give valuable information in investigative work, and yet give results of no apparent clinical significance. There are relatively few procedures for the measurement of hormones or their products which give results of proved clinical significance. By far the most widely used of these is the one which is intended to measure excretion of 17-ketosteroids. Some of the problems connected with this determination have already been discussed in an earlier editorial (4). The many workers who have attempted to improve the method have realized that no matter what procedure is employed for the acid hydrolysis, a very appreciable portion of the conjugated steroids remains unhydrolyzed and therefore unmeasured, and that another portion is transformed to other steroids. For example, dehydroisoandrosterone is largely converted to another steroid which, fortunately, is nevertheless capable of yielding with alkaline *m*-dinitrobenzene a color very similar to that given by dehydroisoandrosterone. Furthermore, the indigoid pigments for which some methods allow correction by subtraction, may very well be derived in part from the same type of steroid which is measured with the Zimmermann reagent. The method measures at best only an approximation of the sum of many different 17-kestosteroids, each of which is presumably present in different proportions in different urines, and each of which is in itself (and is derived from) an androgen of different potency. Despite these limitations, the procedure has proved to yield results which can be correlated surprisingly well with clinical findings.

Unfortunately, a less satisfactory degree of correlation with clinical findings has been obtained with procedures designed to measure the formaldehydogenic steroids (5). The Porter-Silber method (6), or a modification which uses 2:4 dinitrophenylhydrazine (7), may prove to give results of greater clinical significance (5), although measurement of formaldehydogenic steroids will probably continue to be desirable in investigative work.

The method for determining urinary estrogens after preliminary fractionation with either counter-current distribution procedures or chromatography is useful for investigative work, but for most clinical work a simpler procedure would be desirable. Although the fluorescence yielded by acid treatment of estrogens is much more intense than that obtained by the nonphenolic steroids, it is not yet clear how useful the results obtained by this technique will be to clinicians.

The ideal method should satisfy certain criteria. It should measure accurately the substance to be determined; that is, it should be specific and reproducible. It should also be rapid, thus permitting early reporting of results with a minimum of labor. It should be simple, so that there will be less chance of error; and preferably, only inexpensive apparatus should be required. It should be economical, especially with respect to labor, and

involve minimum exposure of the worker to toxic agents. The relative emphasis to be placed on these different criteria will depend upon whether the method is to be used primarily for routine clinical work, or for investigative purposes.

Those who develop photometric methods apparently need to be reminded that, apart from specificity, the greatest precision is obtained by a method when the optical density reading is 0.4343. By adjustment of the concentration of the solution to be read, by use of adsorption cells of appropriate thickness, by selection of a suitable wave length, or by measurement of an unknown against a solution of known concentration rather than against a reagent blank, the optical density readings should be kept between 0.2 and 0.7, because the magnitude of the relative analysis error increases enormously beyond these limits (3).

The grossly inadequate performance of many clinical chemical laboratories surveyed by Belk and Sunderman (8) would probably have been just as great had the laboratories been required to measure corticosteroids, estrogens, or 17-ketosteroids. However, the inconsistencies which these observers reported are probably due, not to unsatisfactory methods, but rather to ineffectual application of relatively satisfactory methods, inadequacies of personnel, and errors in calculation and transcription of results. These factors, which are no less important to endocrinologists than to clinicians in general, can be controlled to a large extent by the application of recommendations outlined elsewhere (3).

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Questions and Answers

(Questions submitted at the 1953 Postgraduate Assembly of the Endocrine Society and the University of Minnesota Medical School)

Answers by Lawson Wilkins, Johns Hopkins Hospital, Baltimore, Md.

QUESTION: What is the mechanism of fat decomposition in infants, so that ketogenic substances are not formed (for instance, in an infant with pyloric stenosis)?

ANSWER: It has been shown that in infants under 6 months of age, ketosis is not prone to occur with starvation or the administration of a ketogenic diet. On the other hand, when ketone bodies are administered, they are excreted through the urine. Older children are especially prone to ketosis during starvation. The cause of the resistance in young infants is not understood. It is stated that the composition of the body fats of infants differs from that in older children, in that there is a higher content of unsaturated fatty acid. It has been suggested that these body fats may be less readily mobilized and metabolized to ketones.

QUESTION: Is delayed bone development ever due to causes other than endocrine?

ANSWER: Yes. There is considerable variation in the rate of epiphyseal development in entirely normal children, so that in later childhood there are frequently deviations of of ± 2 years from the average bone age. Even greater retardation of growth and osseous development may depend upon constitutional factors in individuals who show no evidence of an endocrine disorder. Chronic nutritional disorders and cerebral defects may be accompanied by delayed osseous development. Circulatory impairment in an extremity may cause localized delay in epiphyseal ossification. On the other hand, the most marked degrees of osseous retardation are seen in patients with hypothyroidism or hypopituitarism.

Answers by C. L. Buxton, Columbia-Presbyterian Medical Center, New York, N. Y.

QUESTION: How should a pathologic report on an endometrial biopsy specimen be correlated with clinical findings?

ANSWER: In sterility cases a premenstrual endometrial biopsy specimen is taken to ascertain whether or not the patient has a secretory endometrium, thus indirectly determining if ovulation has occurred. The findings may be correlated with basal body temperature graphs, as confirmatory evidence of ovulation. Variations from normal in the results of these two tests sometimes indicate abnormality of postovulatory luteal activity.

In cases of unexplained menorrhagia, a thorough premenstrual curettage should always be performed instead of an endometrial biopsy, in order to avoid the possibility of missing a small focus of endometrial carcinoma which may be responsible for the symptom.

QUESTION: What is the physiologic basis of "wedge resection" of the ovary in the so-called Stein-Leventhal syndrome?

ANSWER: The physiologic basis of "wedge resection" for bilateral polycystic ovaries is probably not known. Possibly the resection eliminates some inhibitor substance which has been preventing adequate gonadotropin activity on the part of the pituitary; but this is purely conjectural.

Answers by F. Raymond Keating, Jr., Mayo Clinic, Rochester, Minn.

QUESTION: Is it not wiser to start a patient on small doses of thyroid rather than large ones, and increase the dose after several weeks, if necessary?

ANSWER: The decision as to what the initial dose of thyroid should be in a case of myxedema depends upon many factors, including the state of the kidneys and cardiovascular system, the extent and duration of myxedema and the age of the patient. In patients with profound myxedema of long standing, or accompanied by impaired kidney function, coronary insufficiency or cardiac decompensation, it may be necessary to start with very minute doses and to raise the basal metabolic rate under hospital conditions over a long period of time, involving many weeks of closest supervision. In patients with presumably sound renal and cardiovascular systems, exhibiting mild or moderate myxedema of limited duration, such a time-consuming and costly program is unnecessary, since larger doses of thyroid may be tolerated without difficulty. With the foregoing exceptions, most patients can tolerate 1 to 3 grains of desiccated thyroid (U.S.P.) from the beginning of treatment. Whatever the schedule of dosage selected, extremely close supervision is desirable until the basal metabolic rate is raised to the desired level, the symptoms of myxedema eliminated and the patient stabilized on a maintenance schedule.

QUESTION: Please comment on the treatment of myxedema circumscripta.

ANSWER: Myxedema circumscripta, or localized myxedema, is an unusual cutaneous manifestation of Graves' disease, named because of its histologic resemblance to the skin changes found in hypothyroidism. No effective local treatment is known. It is customary to give iodides over a period of time, in the course of which the lesion sometimes subsides. Whether the treatment helps or not is open to question.

QUESTION: What percentage of patients with pituitary myxedema have an elevated serum cholesterol level?

ANSWER: When anterior pituitary insufficiency is accompanied by the usual stigmata of myxedema, serum cholesterol values are sometimes elevated, but the exact proportion of cases in which this occurs has not been determined. Unfortunately, it has become the custom to consider "pituitary myxedema" synonymous with "anterior pituitary insufficiency," whether or not there are clinical signs of classic myxedema, including such manifestations as skin changes and slowed reflexes. In patients with pituitary insufficiency in whom clinical signs of myxedema are lacking, the serum cholesterol level usually is not elevated.

Answer by Leo T. Samuels, Univ. of Utah College of Medicine, Salt Lake City, Utah

QUESTION: Please explain the factors affecting the solubility product of calcium and phosphorus.

ANSWER: An answer to this question is complicated, both because of the number of factors involved and because of the methods of analysis ordinarily used in determining these elements in body fluids.

If one is dealing with a system in which the concentrations of calcium ion and phosphate ion are known, the factors which will affect the solubility product are the concentrations of these two ions, the concentration of hydrogen ion (pH), the concentration of ions such as ^{-}Fl and $^{=}CO_3$ which will form complex crystals, and total ionic strength. The effect will not be simple, since the number of possible types of ion and precipitable salt is large. Actually, however, at any given pH, only a few of these possible components will exist in significant concentrations.

Ordinarily, in biologic fluids total calcium, and not calcium ion, is determined. Under these conditions the term "solubility product" becomes meaningless, since calcium is present as a number of relatively un-ionized salts of substances such as polyvalent acids (proteins, citrate, etc.), and complexes with polyhydroxy compounds, the concentrations of which are usually unknown. No rigid rules can, therefore, be drawn from measurements in biologic systems. In urine, the simplest of such systems, one can apply the broad general concept that deposition is favored by a high calcium×phosphate product and high pH (low acidity).

Answer by Lewis M. Hurxthal, Lahey Clinic, Boston, Mass.

QUESTION: How do you treat juvenile Fröhlich's syndrome with obesity?

ANSWER: It is assumed that the term juvenile Fröhlich's syndrome with obesity means that there is a true hypopituitarism, the commonest causes of which are suprasellar cyst or craniopharyngioma, intrasellar chromophobe pituitary tumor or some post-infectious abnormality of the anterior pituitary. Since the condition in most pubescent fat boys is generally called Fröhlich's syndrome, it is important to differentiate between the two. In order to make a diagnosis of Fröhlich's syndrome, it would appear that there should be definite evidence of retardation of growth, both from the patient's actual height as compared to normal standards, allowing for familial tendencies to short stature, and from school records. Roentgenograms of the skull should be taken, the sella measured, and suprasellar calcification looked for. The bone age is usually retarded compared to the normal development, but in the juvenile age period there should be a lag of at least two years behind the normal average. The usual elevation of the level of fasting inorganic serum phosphorus should not have occurred, particularly before the age of 10.

If a true Fröhlich's syndrome (hypopituitarism with obesity) exists, the child should be treated with testosterone sublingually in doses up to 20 milligrams a day. This may stimulate the growth. Growth hormone is not a feasible treatment at the present time. In some instances in which there is evidence of thyroid deficiency, 1 grain of desiccated thyroid daily may be added. As a rule these children do not need cortisone or desoxycorticosterone acetate, since there is very little disturbance of sodium and potassium metabolism. If, on the other hand, the condition is a simple question of pubescent obesity, dietary measures alone are indicated. There is some difference of opinion as to whether testosterone should be given before the usual age of puberty or whether one should wait until that time. If panhypopituitarism is present, it is not likely that with this amount of testosterone there will be premature closing of the epiphyses. Such a child will undoubtedly not reach his normal height by any method of treatment we have today.

The Endocrine Society

THE 1954 ANNUAL MEETING

The Thirty-sixth Annual Meeting of The Endocrine Society will be held in the Sir Francis Drake Hotel, San Francisco, California, Thursday, Friday and Saturday, June 17–18–19, 1954.

Dr. Roberto F. Escamilla, 655 Sutter Street, San Francisco, California, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Sir Francis Drake Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 18, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

Hotel reservations may be made through the San Francisco Convention and Visitors' Bureau, Room 300, Civic Auditorium, San Francisco, California. They should be accompanied by the official application form which has appeared weekly in the J.A.M.A. since December 1953.

Arrangements have been made for members of this society who do not belong to the AMA to be assigned rooms during the days of the meeting of the Endocrine Society. Applications from nonmembers of the AMA should specify dates of arrival and departure and be accompanied by a deposit of $10.00 per room.

Confirmations will not be sent until after February 1, 1954, but *applications should be made promptly* in order to insure the reservation.

1954 AWARD AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000 may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval

from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected in 1949. The Fellowship in 1950 was awarded to Dr. Lawrence E. Shulman and in 1952 to Dr. John C. Laidlaw. In 1954 it will be in the amount of $5,000.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

This Fellowship will not be awarded in 1954 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1955.

Award

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman; in 1953 the Award was presented to Drs. Sidney Roberts and Clara Szego. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1954.*

The American Goiter Association

THE 1954 ANNUAL MEETING

The annual meeting will be held at the Somerset Hotel, Boston, Massachusetts, April 29, 30 and May 1, 1954. It is suggested that you make your hotel reservations by writing Mr. Harry Hoghn, Executive Assistant Manager, The Somerset, 400 Commonwealth Avenue, Boston 15, Massachusetts.

JOHN C. MCCLINTOCK, M.D.,
Corresponding and Recording Secretary

PROGRAM

THURSDAY, April 29, 1954

9:00 A.M.

1. Factors Governing the Development of the Chick Embryo Thyroid. II. Chronology of the Synthesis of Iodinated Compounds Studied by Chromatographic Analysis.
   J. B. Trunnell and *Paul Wade*, Houston, Texas
2. Hydrolysis of Thyroglobulin by an Intrinsic Enzyme System.
   J. B. Alpers, Jacob Robbins and *J. E. Rall*, New York, N. Y.
3. Metabolic Determinants of the Iodide Concentrating Capacity of Surviving Thyroid Slices.
   Norbert Freinkel and *Sidney H. Ingbar*, Boston, Massachusetts
4. Influence of Various Factors on the Uptake of Iodide by the Thyroid.
   D. Ward Slingerland, Boston, Massachusetts.
5. *In Vitro* Studies of the Intact Thyroid Gland.
   A. L. Botkin, C. D. Eskelson, H. E. Firsheim and *H. F. Jensen*, Fort Knox, Kentucky
6. Studies on the Thyroidal Uptake of Astatine in the Rat.
   C. J. Shellabarger and *John T. Godwin*, Upton, Long Island, New York
7. The Accumulation and Destructive Action of Astatine^{211} (EKA-Iodine) in the Thyroid Gland of Rats and Monkeys.
   J. G. Hamilton, Berkeley, California
8. The Diagnostic Use of I^{132}.
   E. M. Hanbury, Jr., L. G. Stang, W. D. Tucker and *J. E. Rall*, New York, N. Y.

2:00 P.M.

9. Histologic Effects of Various Types of Ionizing Irradiation on Normal and Hyperplastic Human Thyroid Glands.
   Stuart Lindsay, Morris E. Daily and *Earl R. Miller*, San Francisco, California
10. A Study of Iodinated Compounds in the Serum, the Disappearance of Radioactive Iodine from the Thyroid, and the Clinical Response in Patients Treated with Radioactive Iodine.
    Richard S. Benua, and *Brown M. Dobyns*, Cleveland, Ohio

11. Dynamic Studies of Iodine Metabolism in Certain Patients with Goiter.
John B. Stanbury, Kalervo Ohela and *Rosalind Pitt-Rivers*, Boston, Massachusetts
12. High Dosage of Propyl Thiouracil in the Treatment of Hyperthyroidism.
Elmer C. Bartels, Boston, Massachusetts
13. Studies on the Metabolism of Thyrotropic Hormone in the Rat.
Savino A. D'Angelo, Philadelphia, Pennsylvania
14. Induction and Characteristics of Thyrotrope Secreting Pituitary Tumors.
J. Furth, J. N. Dent and *W. T. Burnett*, Oak Ridge, Tennessee
15. The Uptake of Radioactive Iodine in the Thyroid of Patients with Impaired Liver Function.
Richard Mueller, Richard S. Benua, Charles C. Brausch, Eugene Z. Hirsch and *Brown M. Dobyns*, Cleveland, Ohio
16. Evidence for Abnormal Iodine Metabolism in Epidemic Hemorrhagic Fever: A Preliminary Report.
Richard E. Ogborn, Washington, D. C.

FRIDAY, April 30, 1954

9:00 A.M.

17. *Presidential Address.*
Merrill N. Foote, Brooklyn, N. Y.
18. *Van Meter Prize Award.*
19. The Circulating Thyroid Hormone-Plasma Protein Complex in Euthyroid and Hyperthyroid Subjects: Differences in Rate of Disappearance in Dogs and "Uptake" by Rat Diaphragm.
Milton W. Hamolsky and *A. Stone Freedberg*, Boston, Massachusetts
20. Dynamic Analysis of Iodine Metabolism in Four Normal Subjects.
F. C. Hickey and *G. L. Brownell*, Boston, Massachusetts
21. The Utilization of Carbon^{14} Labeled Tyrosine by Thyroid Slices in the *In Vitro* Synthesis of Thyroxine.
Brown M. Dobyns, Shirley R. Barry, Lois A. Wilson, Ivan D. Frantz and *Robert B. Loftfield*, Cleveland, Ohio
22. Radioiodine Studies in Patients with Chronic Renal Disease Dialyzed on the Artificial Kidney.
David V. Becker and *Lamont E. Danzig*, Fort Sam Houston, Texas
23. Renal Function and I^{131} Clearance in Untreated Hyperthyroidism and Myxedema with Observations on the Effects of Triiodothyronine in Myxedema.
Charles J. Hlad and *Neal S. Bricker*, Denver, Colorado

2:00 P.M.

24. Biological Effects of Triiodothyronine in Human Subjects.
Paul Starr and *Ruth Liebhold-Schueck*, Los Angeles, California
25. Functional Properties of Thyroidal Tissue in Fish.
Aubrey Gorbman, New York, N. Y.
26. Calcium as a Goitrogen.
Selwyn Taylor, London, England
27. Cabbages and Turnips as a Cause of Endemic or of Epidemic Goiter in Man and in Farm Animals. The Growth of a Legend.
Isidor Greenwald, New York, N. Y.

28. The Experimental Production of Thyroid Tumors by Alternating Hyperplasia and Involution.
Leo M. Zimmerman, P. Shubik, R. Baserga, A. C. Ritchie and *L. Jacques,* Chicago, Illinois
29. A Method for Increasing the Accuracy of the Radioiodine Uptake in the Diagnosis of Thyroid Disease by Means of Oral Thyroid Medication.
Monte A. Greer and *G. E. Smith,* Bethesda, Maryland
30. Studies on Changes in Nitrogen Excretion during Stressful Life Experiences and their Relation to Thyroid Function.
Basil S. Hetzel, William W. Schottstaedt, William J. Grace and *Harold G. Wolff,* New York, N. Y.

SATURDAY, May 1, 1954

9:00 A.M.

31. The Effect of Thyroid Upon Serum Lipoproteins and Cholesterol.
Beverly Strisower, John W. Gofman, Elmer Galioni, Joshua H. Rubinger and *Alexander Simon,* Berkeley, California
32. The Occurrence of Myotonia in Two Patients Following Thyroidectomy for Hyperthyroidism.
K. R. Crispell and *William Parson,* Charlottesville, Virginia
33. Quantitative Measurements of Radioiodine Retention in Thyroid Carcinoma.
R. M. Cunningham, Gwen Hilton and *E. E. Pochin,* London, England
34. Cancer of the Thyroid.
William V. McDermott, Jr., Winfield S. Morgan and *Edward Hamlin, Jr.,* Boston, Massachusetts
35. Diffuse Anaplastic Carcinoma of Thyroid; Re-evaluation of Postoperative Results.
Ralph Lev, O. H. Beahrs and *E. S. Judd,* Rochester, Minnesota
36. Invasive Papillary Cancer of the Thyroid.
Edgar L. Frazell and *Benedict J. Duffy,* New York, N. Y.
37. Characteristics and Behavior of Thyroid Cancer.
Lawrence W. Sloan, New York, N. Y.
38. Transplant of Thyroid Gland using Vascular Pedicles; Case Report with One Year Follow-Up.
Julian A. Sterling, Philadelphia, Pennsylvania

THE AMERICAN GERIATRICS SOCIETY

The 1954 Annual Meeting of the American Geriatrics Society will be held at the Hotel Fairmont in San Francisco, California, just preceding the meeting of the American Medical Association. The scientific sessions of the meeting will begin Thursday afternoon, June 17, and continue through Saturday morning, June 19.